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Water Quality Criteria for Disperse Red 9

FINAL REPORT

Kowetha A. Davidson
Patricia S. Hovatter

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The available data on the environmental fate, aquatic toxicity, and mammalian toxicity of Disperse Red 9 (1-methylaminoanthraquinone), an organic dye used in pyrotechnic smoke signals, were reviewed. The USEPA guidelines were used in an attempt to generate water quality criteria for the protection of aquatic life and its uses and of human health.

Disperse Red 9 is insoluble in water and exhibits negligible volatility, indicating that environmental dispersal should be minimal. An environmental fate model predicts that the dye would primarily accumulate in water. Limited information is available concerning the transport, transformation, or

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degradation of Disperse Red 9 in the environment. The dye will undergo photodecomposition. Transformation during combustion is by oxidation, with the dye being partially demethylated to 1-aminoathraquinone and with some rearrangement to 2-aminoanthraquinone, indicating possible increased biological activity.

No data are available concerning the toxic effects of Disperse Red 9 on aquatic organisms; therefore, a Criterion Maximum Concentration and a Criterion Continuous Concentration cannot be calculated. Toxicity studies following the USEPA guidelines are recommended.

Although Disperse Red 9 is absorbed from the gastrointestinal tract, it has only mild acute and subchronic oral toxic effects in laboratory animals. It has no dermal toxicity in laboratory animals, but in humans it causes dermal irritation and delayed hypersensitivity reactions. Disperse Red 9 causes mild toxic effects in humans exposed orally or by inhalation. Acute inhalation exposure in rodent and nonrodent species causes nasal irritation, salivation, gagging, regurgitation, dyspnea, and death, depending on the dose and duration of exposure. The LCt_{50}'s for inhalation of Disperse Red 9 range from 352,667 to 815,013 $mg \cdot min/m^3$ in studies involving guinea pigs, rats, rabbits, dogs, and monkeys.

In genotoxicity tests, Disperse Red 9 is negative in Salmonella typhimurium, positive in mouse lymphoma cells in the presence and absence of S9, and positive in unscheduled DNA synthesis assays only in the presence of S9. Disperse Red 9 is considered to be inactive in the dominant lethal assay in mice and in rats.

Disperse Red 9 applied topically is not active as a complete carcinogen nor as a tumor initiator in the SENCAR Mouse Bioassay System (two-stage mouse skin carcinogenesis system). The evidence that Disperse Red 9 is carcinogenic when administered by the oral and inhalation routes was inconclusive.

Because non-threshold and threshold toxicity data were insufficient, a water quality criterion for the protection of human health could not be calculated according to the USEPA guidelines.

ORNL-6356

WATER QUALITY CRITERIA FOR DISPERSE RED 9

FINAL REPORT

Kowetha A. Davidson
Patricia S. Hovatter

Chemical Effects Information Task Group
Information Research and Analysis Section
Biology Division

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EXECUTIVE SUMMARY

Disperse Red 9 (1-methylaminoanthraquinone) is an organic dye prepared by an alkyl amination reaction. It is used by the military in red- and violet-colored smoke grenades as a means of communication. It is also used as a coloring agent for synthetic fabrics and plastics.

The environmental release of Disperse Red 9 may occur during manufacturing and during formulation and loading of smoke grenades, or upon detonation of the grenades during training and testing operations. The pyrotechnic unit consists of the dye mixture, oxidizer, fuel, coolant, and diatomaceous earth as a binder. Upon detonation of the grenade, heat from the burning fuel causes the dye to volatilize; the vapor then condenses outside the unit, forming a smoke cloud. Combustion products may enter aquatic environments directly as fallout, or indirectly by leaching from soils or in runoff.

Disperse Red 9 is insoluble in water and, consequently, may occur as a suspensoid or emulsoid. An environmental fate model predicts that the dye would primarily accumulate in water vs. soil, air, sediment, or biota. Limited information is available concerning the transport, transformation, or degradation of Disperse Red 9 in the environment. The dye will undergo photodecomposition in the environment. During combustion, however, 5 to 10 percent of the dye is oxidized. Decomposition occurs by breakage of side chains, but not by ring openings. The major chemical effect of combustion is the partial demethylation to 1-aminoanthraquinone with some rearrangement to 2-aminoanthraquinone, indicating possible increased biological activity.

No data are available concerning the toxic effects of Disperse Red 9 on aquatic organisms. Toxicity studies with similar dyes indicate that pH may alter the toxic effect of effluents by influencing the degree of ionization and the site of action. Because of insufficient data, a Criterion Maximum Concentration and a Criterion Continuous Concentration could not be calculated. Consequently, it is recommended that the toxicity studies required by the USEPA guidelines (Stephan et al. 1985) be performed.

Very few data are available on toxic effects of Disperse Red 9 in mammals. Approximately 84 percent of the dose of Disperse Red 9 administered orally to sheep may be absorbed from the gastrointestinal tract. Approximately 27 percent of the administered dose can be recovered in urine as colored metabolites unconjugated or conjugated with glucuronic acid; approximately 16 percent of the administered dose appears in feces as unmetabolized Disperse Red 9. Therefore, more than 50 percent of the dose is probably noncolored metabolites.

Colored metabolites recovered in the urine of sheep are conjugated or unconjugated forms of 1-aminoanthraquinone, 2-hydroxy-1-aminoanthraquinone, and 4-hydroxy-1-aminoanthraquinone. Small amounts of conjugated

and unconjugated metabolites are found in bile, liver, and kidney, but only conjugated metabolites are found in milk.

The absence of colored substances in the urine of animals topically exposed to Disperse Red 9 suggests that it is not absorbed from the skin. Disperse Red 9 administered by inhalation is not retained in the respiratory tract, but the appearance of colored substances in the urines suggest that it may be absorbed from the lungs.

In laboratory animals, Disperse Red 9 is only slightly toxic by the oral route. The acute toxic and lethal dose in dogs is greater than 8 g/kg; 2 g/kg/day for 5 days is only mildly toxic in mice and rats. Disperse Red 9 applied to the skin of rabbits is not toxic or irritating, and it causes only temporary discomfort when applied to the eyes.

The effects of acute inhalation exposure to Disperse Red 9 are more severe than effects of oral administration. Symptoms caused by inhalation include nasal irritation, salivation, gagging, regurgitation, dyspnea, and death, depending on the dose and duration of exposure. The LCt_{50}'s in rats, guinea pigs, rabbits, dogs, and monkeys range from 352,667 to 815,013 $mg \cdot min/m^3$. Based on LCt_{50}'s, the decreasing order of sensitivity is guinea pigs > dogs > rats > rabbits > monkeys; based on the mortality data, however, goats and swine are less sensitive than monkeys.

Chronic oral exposure of laboratory animals to Disperse Red 9 causes only mild toxic effects. Chronic inhalation of a smoke mixture containing Disperse Red 9, Solvent Yellow 33, and Solvent Green 3 causes nonspecific damage to the respiratory tract.

In mutagenicity tests, Disperse Red 9 is negative in Salmonella typhimurium, positive in mouse lymphoma cells in both the presence and absence of S9, and active in the unscheduled DNA synthesis assay, but only in the presence of S9. In the dominant lethal assay in rats, but not in mice, Disperse Red 9 causes a statistically significant increase in the proportion of dead implants in the week 4 mating, possibly due to the unusually low values in controls. The dye is considered to be negative in the dominant lethal assay in both mice and rats.

Disperse Red 9 applied topically is not active as a complete carcinogen nor as a tumor initiator in the SENCAR Mouse Bioassay System (two-stage mouse skin carcinogenesis system). Although one unusual neoplastic lesion appeared in one animal administered Disperse Red 9 by intragastric intubation, the dye was not considered carcinogenic in that test. Neoplastic lesions were also induced when Disperse Red 9 was administered by inhalation to laboratory animals along with Solvent Yellow 33 and Solvent Green 3. Nevertheless, the evidence that Disperse Red 9 is carcinogenic is inconclusive.

In humans, dermal contact with Disperse Red 9 causes irritation and delayed hypersensitivity reactions in the skin. Acute oral and inhalation

exposure causes only mild toxic effects. The toxicity rating assigned to Disperse Red 9 is "1."

Because sufficient data were not available, the USEPA guidelines could not be used to calculate a water quality criterion for the protection of human health. Additional research was recommended to fill data gaps.

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1. INTRODUCTION

Disperse Red 9 (1-methylaminoanthraquinone) is an organic dye used by the military in M18 colored smoke grenades (red and violet) as a means of communication. Colored smokes can be used to identify specific ground positions and coordinate assault operations (Cichowicz and Wentsel 1983). Anthraquinone disperse dyes are also used as coloring agents for industrial synthetic fabrics (Chung and Farris 1979) and plastics (Webber 1979). Disperse Red 9 is not certified for use in cosmetics, food, or drugs (Dacre et al. 1979).

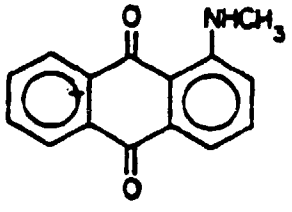
The pyrotechnic composition of colored smoke grenades consists of the dye mixture, an oxidizer, fuel, coolant, and diatomaceous earth as a binder. Each grenade contains approximately 320 g of the dye mixture, which is formulated at Aberdeen Proving Ground, Maryland (Henderson et al. 1985). The cooling agent is used to prevent excessive decomposition of the organic dye due to heat produced by the fuel. Upon detonation of the grenade, heat from the burning fuel causes the dye to volatilize; the vapor then condenses outside the pyrotechnic, thereby producing smoke. The burning time is adjusted by the proportion of fuel and oxidizer and by the use of the cooling agent (Cichowicz and Wentsel 1983). The properties of dyes that make them suitable for use as colored smokes are: (1) rapid volatization at 400 to 500°C; (2) minimum decomposition; (3) molecular weight not > 450; and (4) purity of color and stability of the smoke condensate in air (Shidlovskiy 1964, as reported in Chin and Borer 1983).

The parent compound of Disperse Red 9 is 9,10-anthraquinone; many of the natural and synthetic derivatives of 9,10-anthraquinone are active in mutagenicity assays (Brown and Brown 1976, Brown 1980, Liberman et al. 1982, Sigman et al. 1985), suggesting that the compounds may also have carcinogenic potential (Sigman et al. 1985). Consequently, the Army Armament Research and Development Command at Aberdeen Proving Ground, Maryland, has been conducting feasibility studies on the replacement of Disperse Red 9 in colored smoke grenades with dyes less likely to have mutagenic or carcinogenic activity (Vigus and Deiner 1981). The three replacement dyes currently under investigation are Sudan R (Q-methoxyphenyl-azo-β-naphthol), Macrolex Red 1069, and Amoplast Red PC (Smith and Stewart 1982). Presently, insufficient toxicological information is available to assess the health effects of these dyes.

Currently, however, the production and use of red- and violet-colored smoke grenades could result in human and environmental exposure to Disperse Red 9 and its combustion products. Consequently, the objectives of this report are to review the available literature concerning the environmental fate, aquatic toxicity, and mammalian toxicity of this dye in order to generate water quality criteria according to current USEPA guidelines. The methodology for deriving criteria to protect aquatic life and its uses (Stephan et al. 1985) is summarized in Appendix A; the methodology for deriving criteria to protect human health is summarized in Appendix B (USEPA 1980).

1.1 PHYSICAL AND CHEMICAL PROPERTIES

The physical and chemical properties of Disperse Red 9 are listed as follows:

CAS registry No.:	82-38-2
Color index (CI) No.:	60505 (Chung and Farris 1979)
Chemical name:	Disperse Red 9
Synonym, trade names:	1-(methylamino)-9,10-anthracenedione (9 C.I.) 1-(methylamino)-anthraquinone, C.I. Disperse Red 9, C.I. Solvent Red 111, Amaplast Red AAP, Calco Oil Red ZMQ, Duranol Red GN, Macro-lex Red G, Orient Oil Red 330 [MEDLARS II (CHEMLINE) 1986]
Structural formula:	
Molecular formula:	$C_{15}H_{11}NO_2$
Molecular weight:	237.26 (Weast and Astle 1985)
Physical state:	Yellow-red needles (Weast and Astle 1985)
Melting point (°C):	170 (Weast and Astle 1985)
Boiling point (°C):	310, calculated from vapor pressure equation (Deiner 1982)
Density (g/mL):	0.35 ± 0.15 (Cichowicz and Wentsel 1983)
Solubility:	Insoluble in water (< 0.0002 g/100 mL at 25°C, 0.00055 g/100 mL at 80°C); soluble in acetone, alcohol (14.96 g/L at 60°C), chloroform, cellosolve; slightly soluble in benzene, carbon tetrachloride, hot dilute hydrochloric

	acid, glacial acetic acid, and fuming sulfuric acid (Dacre et al. 1979, Cichowicz and Wentsel 1983, Marrs et al. 1984).
Heat of vaporization (kcal/mol at 25°C and 1 atm):	28.4 ± 0.07 (Benyon and Nicholson 1956, as reported in Cichowicz and Wentsel 1983)
Entropy of vaporization (cal/mol/°C):	52.87 ± 0.30 (Bradley et al. 1956, as reported in Cichowicz and Wentsel 1983)
Heat of sublimation (kcal/mol):	30.85 (Bradley et al. 1956, as reported in Cichowicz and Wentsel 1983)
Energy of activation for volatilization (kcal/mol at 297-376°C):	19.6 (Cichowicz and Wentsel 1983)
Vapor pressure (log P (cm Hg) = a/T + b):	a = 6740, b = 13.435, T = absolute temperature (Bradley et al. 1956, as reported in Cichowicz and Wentsel 1983)
Explosibility index (dust):	1.1 (moderate to strong explosion) (Dorsett and Nagy 1968)
UV Absorption (nm):	508 (Chung and Farris 1979)
Other:	Sensitive to UV radiation; exhibits changes in presence of O_2 and oxidizing agents (Dacre et al. 1979)

1.2 MANUFACTURING AND ANALYTICAL TECHNIQUES

Disperse Red 9 is prepared by an alkyl amination reaction. Methylamine is treated under pressure with 1-anthraquinonesulfonic acid or 1-chloroanthraquinone in the presence of an oxidizing agent. The primary U.S. producer as of 1984 was the American Cyanamid Company, New Jersey (SRI 1984). U.S. production in 1979 was > 9,922 lb (USITC 1980a, as reported in Sigman et al. 1985) and importation was approximately 19,619 lb (USITC 1980b, as reported in Sigman et al. 1985). The average annual use of Disperse Red 9 by the U.S. Army between 1965 and 1975 was 76,300 lb/yr (Burrows 1977). According to Cichowicz and Wentsel (1983), the U.S. Army currently has the dye stockpiled and uses 100,000 lb/yr (8,330 lb/month), which is approximately 13 percent of the civilian production capability of 750,000 lb/yr. The full mobilization rate of use would be 17,300 lb/month (Kitchens et al. 1978).

Red and violet smoke grenades are formulated and loaded at the Pine Bluff Arsenal in Arkansas by means of the Glatt Mixing Process, which

started production in 1984. A fluidized bed granulator combines the three operations of mixing, granulation, and drying. This technique improves efficiency and provides for material containment, thereby reducing worker exposure to dust and the pollutant discharge of acetone (Garcia et al. 1982). The formulation of the red smoke grenade is as follows: 40 percent Disperse Red 9 (red smoke mix), 26 percent potassium chlorate (oxidizer), 25 percent sodium bicarbonate (coolant), and 9 percent sulfur (fuel) (Military Specification 1971). Violet smoke grenades have the following formulation: 42 percent violet smoke mix (20 percent Disperse Red 9 and 80 percent 1,4-diamino-2,3-dihydroanthraquinone), 25 percent potassium chlorate, 24 percent sodium bicarbonate, and 9 percent sulfur (Military Specification 1970).

Disperse Red 9 and mixtures of anthraquinone dyes can be readily separated by thin-layer chromatography using a variety of solvents (Egerton et al. 1967, as reported in Dacre et al. 1979). High-pressure liquid chromatography has been adapted to separate and identify Disperse Red 9 (Gosnell 1976, as reported in Dacre et al. 1979). Components of colored smoke mix can be identified by various methods, dependent upon the solubility and volatility of the compounds. These techniques include gas chromatography, combined gas chromatography/mass spectrometry, nuclear magnetic resonance spectroscopy, and fluorescence spectrometry (Rubin and Buchanan 1983).

Rubin, Buchanan, and Olerich (1982) conducted a preparative-scale separation of the Disperse Red 9 smoke mix from its impurities. The red smoke mixture was separated into three fractions using vacuum sublimation. Thin-layer chromatography indicated that the first fraction, 11.7 percent of the mixture, was composed primarily of Disperse Red 9, with several impurities. The major contaminant was identified as aminoanthraquinone using combined gas chromatography with a flame ionization detector and mass spectrometry. Other impurities at < 1 percent of the mixture were identified as azobenzene, azoxybenzene, aminobiphenyl, and phenyldiazobenzene. The second fraction, 73.6 percent of the mixture, contained 98 percent pure Disperse Red 9. The third fraction, 12.7 percent of the mixture, was the residue, a nonvolatile black powder containing carbon, hydrogen, and oxygen with hydroxyl groups.

The particle size distribution of the colored smoke mixture can be determined using a Bahco Microparticle Classifier. Rubin and Buchanan (1983) measured the particle size distribution of Disperse Red 9 by drying 12 g of the dye at 105 to 110°C, weighing the sample on an analytical balance, and then sieving it through a 100-mesh screen. The dye mixture was found to contain 25 percent particles < 10 μm, 40 percent particles > 150 μm, and 35 percent particles with an aerodynamic median diameter of 32 μm. Particles < 10 μm in diameter are considered respirable. Particles > 150 μm are too large to pass through the 100-mesh screen of the Bahco Classifier.

2. ENVIRONMENTAL EFFECTS AND FATE

2.1 ABIOTIC ENVIRONMENTAL EFFECTS

No information was found in the literature concerning the abiotic effects of Disperse Red 9.

2.2 ENVIRONMENTAL FATE

2.2.1 Sources and Transport

The environmental release of Disperse Red 9 may occur either during manufacturing and during formulation and loading of smoke grenades, or upon detonation of the M18 grenades during training and testing operations. Combustion products may enter the aquatic environment directly as fallout, by runoff, or by leaching from soils, depending on the solubility of the product, but impact is usually local in nature, within 10 to 15 km downwind of the site (Cichowicz and Wentsel 1983). Dacre et al. (1979) reported that, at the Pine Bluff Arsenal, only surface areas adjacent to manufacture and test sites show recognizable soil and water coloration.

Disperse Red 9 exhibits limited solubility in water and negligible volatility; consequently, dispersal should be minimal. Its low solubility in water indicates that it should occur in the form of a suspensoid or emulsoid. It is unknown, however, whether Disperse Red 9 is degraded, transformed, or transported as particulates (Dacre et al. 1979).

The Environmental Technology Division of the Chemical Research, Development and Evaluation Center at Aberdeen Proving Ground developed an environmental fate model to predict where the dye would concentrate (air, water, soil, sediment, or biota) (Cichowicz and Wentsel 1983). The model used chemical and physical data related to the potentiality of its release, such as: molecular weight, environmental temperature, water solubility, vapor pressure, log octanol/water (K_{ow}) partition coefficient, and amount released in moles. Hypothetical high and low values, 3.0 and 1.5, respectively, for the log K_{ow} partition coefficient were used in order to cover the range where the actual value may fall. Results of the model, presented in Table 1, indicated that Disperse Red 9 should primarily accumulate in water (59.6 percent at log K_{ow} 3.0 and 99.6 percent at log K_{ow} 1.5) (Cichowicz and Wentsel 1983).

TABLE 1. ENVIRONMENTAL FATE MODEL FOR 1-METHYLAMINOANTHRAQUINONE[a]

Impact		Percent of MAAQ in Each Phase[b]				
		Air	Water	Soil	Sediment	Biota
Molecular weight	237.1 g/mol					
Temperature	25°C					
Water solubility	50 μg/L	32.6	59.6	1.4	6.4	< 0.1
Vapor pressure	0.0002 Pa					
Log K_{ow}	3.00					
Molecular weight	237.1 g/mol					
Temperature	25°C					
Water solubility	50 μg/L	0.0	99.6	0.07	0.34	< 0.1
Vapor pressure	0.0002 Pa					
Log K_{ow}	1.5					

a. From Cichowicz and Wentsel 1983.
b. MAAQ = 1-methylaminoanthraquinone (Disperse Red 9).

2.2.2 Degradation and Combustion Products

No specific information was found in the literature concerning the pathways involved in the degradation of Disperse Red 9. The dye, however, will undergo photodecomposition, with the rate depending on environmental conditions (Kitchens et al. 1978). Anthraquinones, however, are readily reduced to their corresponding diols (Dacre et al. 1979). Deiner (1982) states that the route of degradation during dissemination of the colored smoke grenade is by oxidation.

Several investigators have studied the effluents produced by the combustion of the red smoke mixture (Rubin et al. 1983, Rubin, Buchanan, and Moneyhun 1982, Chin and Borer 1982, 1983, Chin et al. 1984). Separation of the particulate phase by liquid chromatography on silica gel and subsequent analysis using gas chromatography and mass spectrometry found that 86 percent of the chloroform-soluble portion of the combusted smoke is Disperse Red 9. Ten percent of the smoke is 1-aminoanthraquinone and 2-aminoanthraquinone, a tenfold increase over the uncombusted mixture. This chemical transformation may indicate possible increased biological activity (Rubin, Buchanan, and Moneyhun 1982). The major constituents of the vapor phase of the chloroform-soluble portion are carbon disulfide, toluene, C_2-benzenes, styrene, chloromethylbenzene, and naphthalene. Minor constituents include aliphatic and aromatic hydrocarbons, alkylbenzenes, and thiophene. The nonvolatile and/or chloroform-insoluble portion of the combusted smoke contains sodium and potassium chlorides, elemental sulfur, and carbonaceous matter (Rubin et al. 1983).

The combustion products are a result of thermal decomposition, thermal rearrangement of the parent dye, and uncombusted impurities (Chin et al. 1984). The major chemical effect of combustion is the partial demethylation of Disperse Red 9 to 1-aminoanthraquinone, with some rearrangement to 2-aminoanthraquinone (Rubin et al. 1983). However, at the normal function temperature of 440-610°C only side chain breaks occur with no ring openings of the dye (Chin and Borer 1983). Nuclear magnetic resonance (NMR) studies indicate that 5 to 10 percent of the dye decomposes (Chin and Borer 1982).

2.3 SUMMARY

Disperse Red 9 may be released into the environment either during manufacturing and during formulation and loading of smoke grenades, or upon detonation of M18 colored smoke grenades during training and testing operations. Its low solubility in water and negligible volatility indicate that dispersal should be minimal. An environmental fate model speculates that the primary accumulation should occur in water, probably in the form of a suspensoid or emulsoid. Disperse Red 9 will photodecompose, with the rate of decomposition dependent on surrounding conditions. During dissemination approximately 5 to 10 percent of the dye decomposes by oxidation. Upon combustion, Disperse Red 9 is partially demethylated to 1-aminoanthraquinone, with some rearrangement to 2-aminoanthraquinone, indicating possible increased biological activity.

3. AQUATIC TOXICOLOGY

3.1 ACUTE TOXICITY TO ANIMALS

No information was found in the literature concerning the acute toxicity of Disperse Red 9 to aquatic organisms.

3.2 CHRONIC TOXICITY TO ANIMALS

No information was found in the literature concerning the chronic toxicity of Disperse Red 9 to aquatic organisms.

3.3 TOXICITY TO MICROORGANISMS AND PLANTS

No information was found in the literature concerning the toxicity of Disperse Red 9 to microorganisms and plants.

3.4 BIOACCUMULATION

No information was found in the literature concerning the bioaccumulation of Disperse Red 9 by aquatic organisms.

3.5 OTHER DATA

Little et al. (1974) have investigated the acute toxicity of selected commercial dyes to Pimephales promelas (fathead minnow) and found that pH may affect the toxicity by influencing the degree of ionization and the site of action of the dye within the organism. Consequently, if the dye is discharged with other materials that are either acid or alkaline in nature, the toxic effect may be altered.

3.6 SUMMARY

Since no information was found concerning the toxic effects of Disperse Red 9 to aquatic organisms, and since evidences of its current use and potential environmental release are available, it is quite apparent that much research is needed.

4. MAMMALIAN TOXICOLOGY AND HUMAN HEALTH EFFECTS

4.1 PHARMACOKINETICS

4.1.1 Animal Data

Only two studies on the pharmacokinetics of Disperse Red 9 were found in the literature. Martin et al. (1983) administered 50 mg/kg (total dose 2.59 g) of Disperse Red 9, as a dry powder in gelatin capsules, orally to one female sheep. Urine was collected by means of a Foley urinary catheter prior to treatment and every 12 hr for 96 hr after treatment; feces were collected every 24 hr. The colored metabolites of Disperse Red 9 appearing in urine and feces were isolated, identified, and quantitated by thin-layer chromatography, mass spectral and NMR analyses, and visible light spectrophotometry.

The recovery of unmetabolized Disperse Red 9 and colored metabolites in urine and their identities are presented in Table 2. Twelve hours after dosing, 5.72 percent (148.1 mg[1]) of the administered dose was recovered in urine; metabolites conjugated with glucuronic acid accounted for 5.59 percent, and unconjugated metabolites accounted for only 0.13 percent. From 84 to 96 hr, only a small amount was recovered, indicating that urinary excretion of the colored substances was essentially complete. During the entire sampling period, 27.34 percent (708.1 mg) of the administered dose was recovered as colored substances in urine; only 1.48 percent (38.3 mg) was unconjugated, and the remainder was congugates of glucuronic acid.

The primary metabolite, which accounted for more than 70 percent (518.8 mg) of the metabolites recovered in urine, was 2-hydroxy-1-aminoanthraquinone. The second most predominant metabolite, which accounted for 19.6 percent (138.8 mg) of the metabolites recovered, was the glucuronide conjugate of Disperse Red 9. The other metabolites, 1-aminoanthraquinone and 4-hydroxy-1-aminoanthraquinone, were recovered only in trace amounts.

Unmetabolized Disperse Red 9 was the only compound recovered in feces, and this represented 16 percent of the administered dose. Unless it is assumed that Disperse Red 9 is metabolized to noncolored metabolites within the gastrointestinal tract and eliminated in feces, then at least 84 percent of the administered dose was absorbed. As indicated by the presence of colored substances, the gastrointestinal tract and urinary system accounted for elimination of 43.4 percent of the dose administered orally. Therefore, according to Martin et al. (1983) more than 50 percent of Disperse Red 9 was metabolized to non-colored metabolites, which were produced by ring openings or other reactions that destroy the chromogenic properties of the dye.

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[1]The quantity of metabolites was calculated by K. Davidson and is equivalent to milligrams of Disperse Red 9.

TABLE 2. RECOVERY OF COLORED METABOLITES IN THE URINE OF A EWE AFTER ADMINISTERING 2.59 g OF DISPERSE RED 9[a]

	% of Administered Dose Recovered[b]				
Time (hr)	MAAQ[c]	AAQ	4-OH AAQ[d]	2-OH AAQ	TOTALS
12	0.51	0.19	< 0.01	5.02	5.72
24	0.94	0.18	< 0.02	5.61	6.73
36	0.94	0.19	< 0.02	4.16	5.29
48	0.72	0.17	< 0.02	1.55	2.44
60	1.49	0.26	< 0.03	2.14	3.89
72	0.63	0.14	< 0.02	0.96	1.73
84	0.69	0.18	< 0.02	0.52	1.39
96	0.06	0.02	< 0.01	0.07	0.15
Totals	5.98	1.33	< 0.15	20.03	27.34

a. Adapted from Martin et al. 1983.
b. Adjusted by molecular weight to represent % of MAAQ administered.
c. MAAQ = 1-methylaminoanthraquinone (Disperse Red 9); AAQ = aminoanthraquinone; 4-OH AAQ = 4-hydroxy-1-aminoanthraquinone; 2-OH AAQ = 2-hydroxy-1-aminoanthraquinone.
d. Values for 4-OH AAQ reported as < (less than) were not included in the totals.

Martin et al. (1983) also administered 50 mg/kg (2.12 g) orally to a lactating ewe in order to study the distribution of Disperse Red 9 and its metabolites. Milk was collected every 10 hr and venous blood was collected 30 hr posttreatment, at which time the animal was sacrificed for gross necropsy. Brain, kidney, liver, muscle, fat, and a bile sample were taken during necropsy.

The light pink color of the milk indicated that metabolites were present. Unconjugated metabolites were not detected in milk, but glucuronide conjugates of 2-hydroxy-1-aminoanthraquinone (0.003 percent or 107.9 μg) and 4-hydroxy-1-aminoanthraquinone (< 0.002 percent) were found in trace amounts. Thus, within 30 hr, less than 0.005 percent of the administered dose of Disperse Red 9 was metabolized and secreted into milk.

In contrast to milk, unconjugated as well as conjugated metabolites were detected in tissue samples. Only liver, kidney, and bile, however, accumulated metabolites in quantities sufficient to quantitate. Approximately 2 ppm was detected in bile, and 3 ppm was detected in kidney and in liver.

According to Martin et al. (1983) the identity of the colored metabolites established the mechanism by which Disperse Red 9 is metabolized. The major steps were identified as N-demethylation, 2-hydroxylation and

4-hydroxylation of the amino-substituted ring, and conjugation of Disperse Red 9 and its metabolites with glucuronic acid.

The study by Martin et al. (1983) demonstrated that Disperse Red 9 is absorbed from the gastrointestinal tract and distributed to liver, kidney, and bile in very small amounts. Disperse Red 9 is also secreted into milk in trace amounts, and it is eliminated primarily by means of the urinary system. Absorption of Disperse Red 9 from the skin of rabbits was also studied (Martin et al. 1983). The dye, in 95 percent ethanol, was applied to a shaved area on the backs of five male and five female rabbits for 24 hr. Observations for 14 days did not reveal evidence of colored metabolites in the urine nor signs of systemic toxicity. Therefore, Martin et al. (1983) concluded that Disperse Red 9 was not absorbed through the skin in appreciable quantities.

Marrs (1983) demonstrated that Disperse Red 9 was not retained in, but was rapidly cleared from the lungs. Twenty-one Porton female mice, Wistar female rats, and Dunkin-Hartley guinea pigs were exposed to a smoke mixture containing Disperse Red 9 (16 percent), Solvent Yellow 33 (13 percent), and Solvent Green 3 (19 percent) for 30 min at a concentration of 595 mg/m^3. Disperse Red 9 was not found in the lungs of the animals sacrificed between 80 min and 21 days. In another experiment, groups of 10 animals of each species were exposed to 500 mg/m^3 for 1 hr each day for 5 days. As in the previous experiment, Disperse Red 9 was not found in the lungs or taken up by alveolar macrophages between 1 day and 8 weeks.

In a long-term experiment, a total of 400 Porton-strain SPF female mice, 200 Porton Wistar-derived female rats, and 200 Dunkin-Hartley female guinea pigs were exposed to the same smoke mixture for 1 hr per day, 5 days per week for a total of 20 weeks (Marrs et al. 1984). The concentrations were 105.8 mg/m^3 (low dose), 309.6 mg/m^3 (medium dose), and 1012.4 mg/m^3 (high dose), except that the high dose for guinea pigs was 1162.1 mg/m^3. Some of the mice were sacrificed 40 weeks after initiating exposure, and the remainder of the animals were sacrificed 71 weeks after initiating exposure. Disperse Red 9 was not retained in the lungs or found in alveolar macrophages. The means by which Disperse Red 9 was cleared from the lungs was unknown.

4.1.2 Human data

No data were found on the pharmacokinetics of Disperse Red 9 in humans.

4.2 ACUTE TOXICITY

4.2.1 Animal Data

4.2.1.1 Oral Toxicity

According to Dacre et al. (1979), Disperse Red 9 is slightly toxic to rats given 500 mg/day for 3 days. Brusick and Matheson (1978) reported that random bred mice administered Disperse Red 9 in their diet and random bred rats administered the compound by gavage, both at doses of 0.2, 0.67, and 2.0 mg/kg/day for 5 consecutive days, showed only slight signs of toxicity.

Martin et al. (1983) administered 4 g/kg of Disperse Red 9 orally in gelatin capsules to three dogs over an 8-hr period, 8 g/kg to two dogs over a 24-hr period, and gelatin capsules only to two dogs (controls). Treated animals did not show signs of toxicity during a 14-day observation period; weight gain was normal and gastrointestinal problems were absent. Blood chemistry and hematology values of posttreatment blood compared with those of pretreatment blood were found to be within the normal range. Agent-related gross lesions were not found at necropsy. Thus, toxic and lethal doses were greater than 8 g/kg. Although all parameters of toxicity were normal, the presence of colored substances in the urine during the first 48 hr indicated that Disperse Red 9 was absorbed in appreciable quantities from the gastrointestinal tract. A large amount of the dye was also found in the feces.

4.2.1.2 Dermal and Ocular Toxicity

Male and female rabbits, five of each sex, treated topically for 24 hr with a dry powder of Disperse Red 9 (2 g/kg) dissolved in enough 95 percent ethanol to form a paste did not show signs of toxicity or irritation during the 14-day observation period (Martin et al. 1983). Weight loss, edema, and eschar formation were not observed. Because the red dye adhered to the skin, signs of erythema were not evaluated.

Martin et al. (1983) also tested Disperse Red 9 for its ability to photosensitize the skin of rabbits. A saturated solution of Disperse Red 9 in 95 percent ethanol was spotted on the shaved backs of six rabbits. After 30 min, the animals were exposed for 20 min to long-wave ultraviolet light. This procedure was repeated daily for 4 days prior to evaluating the skin for signs of photosensitization.

Gross and histological signs of photosensitization (erythema or edema) were not observed in the areas treated with Disperse Red 9. Martin (1982) also reported that sheep given a single oral dose of Disperse Red 9

(100 mg/kg) and exposed to sunlight for 10 days showed no signs of photosensitization.

Disperse Red 9 (50 mg of the dry powder) placed in the right eye of six rabbits caused slight ocular discomfort immediately after application and conjunctival erythema during the first 12 to 24 hr. The dye did not cause permanent damage to the corneas, conjunctivae, or irises.

4.2.1.3 Inhalation Toxicity

Red smoke mix contains 98 percent Disperse Red 9 and small amounts of 1- and 2-aminoanthraquinone, whereas the combustion products, disseminated by the detonation of red smoke grenades, contain only 86 percent Disperse Red 9, approximately 10 percent 1- and 2-aminoanthraquinone, and other products, including hydrocarbons (Rubin, Buchanan, and Moneyhun 1982). Therefore, inhalation studies include exposure to numerous compounds in addition to Disperse Red 9.

The toxicity of combustion products of nine different pyrotechnics, including a red smoke pyrotechnic unit comprised of 43 parts by weight of Disperse Red 9, was reported by Weeks and Yevich (1963). Red smoke pyrotechnic units were detonated inside static chambers in order to produce smoke with relative concentrations of low, medium, and high. The relationships of the chamber size, number of units fired, particulate concentrations, and relative exposure levels are shown in Table 3. The initial particulate concentration decreased very rapidly, with only 20, 16, and 10 percent of the low, medium, and high exposure levels, respectively, suspended in the chambers 15 min after detonation.

Ten male rats, ten female mice, and five each, male and female guinea pigs were exposed for 1 hr. Animals that survived exposure were observed for signs of toxicity and mortality for 7 days and sacrificed 4 weeks after exposure for histopathological evaluation (Weeks and Yevich 1963).

TABLE 3. RELATIONSHIP OF EXPOSURE LEVEL TO PARTICULATE CONCENTRATION[a]

Exposure level	Chamber size(L)	No. of Charges	Particulate Concentration (mg/L) 3 min	15 min	30 min	45 min
Low	20,000	4	0.20	0.04	0.03	0.00
Medium	700	1	0.62	0.10	0.05	0.00
High	700	3	7.02	0.67	0.16	0.00

a. Adapted from Weeks and Yevich 1963.

All animals exposed to the low dose survived, whereas all animals exposed to the high dose died within 24 hr. Three of ten mice exposed to the medium dose died within 3 days, and the remaining animals survived.

Toxic signs in animals that survived exposure included eye irritation, nasal discharge, gasping, and lethargy. In addition, during the first 3 days after exposure, a decrease in weight gain, which gradually returned to normal, was observed in animals exposed to the medium dose. Only a slight decrease in weight gain was observed in animals exposed to the low dose.

Gross and histopathological evaluation of dead and surviving animals revealed that early effects (up to 24 hr after exposure) were characterized by nonspecific changes in the respiratory tract. In animals exposed to medium and high doses, the nasal passages contained large amounts of particulate matter that adhered to the tracheal mucosa, obliterating the bronchial and bronchiolar tubes. The only microscopic change observed was the presence of alveolar edema. Animals exposed to the low dose were not examined during the first 24 hr.

Late effects (> 24 hr postexposure) included numerous areas of consolidation in the lungs of animals exposed to medium and high doses. Chronic inflammation of the nasal epithelium and hypertrophy and metaplasia of the respiratory mucosa were also noted. Animals exposed to the low dose presented similar but less severe lesions.

Owens and Ward (1974) exposed 7 animal species (monkey, dog, goat, swine, rabbit, rat, guinea pig) to a single dose of combusted red smoke. Groups of 6 or 20 (rats and guinea pigs) animals were placed in a 20,000-L static chamber where grenades (between 1 and 12) were serially fired over a specified period of time in order to obtain a desired concentration x time (Ct). Concentrations ranged from 1,450 to 17,946 mg/m^3, the exposure time ranged from 10 to 240 min, and the Ct's ranged from 179,460 to 1,112,940 $mg \cdot min/m^3$. The animals were observed for 30 days for mortality and signs of toxicity. The LCt_{50} (lethal concentration x time) values were calculated from a Bliss analysis of the mortality response data.

Toxic signs and time of onset after exposure to combusted red smoke are presented in Table 4. All animals showed signs of respiratory tract irritation attributed to nonspecific causes. Salivation and dyspnea (labored respiration) were observed in all species; gagging and regurgitation of a very thick red mucus were observed only in dogs, swine, goats, and monkeys, indicating that these species swallowed a considerable amount of the combustion products. The dark red color of the urine during the first 24 hr after exposure indicated that the dye was absorbed from either the lungs or the gastrointestinal tract.

TABLE 4. TOXICITY AND TIME OF ONSET IN SEVEN SPECIES FOLLOWING INHALATION OF RED SMOKE DISSEMINATED FROM THE M18 GRENADE[a]

	Time of Onset (hr)						
Toxic Signs	Dog	Swine	Goat	Monkey	Rabbit	Rat	G.P.[b]
Nasal irritation	0.25	2	2	2	1.5	0.25	0.25
Salivation	0.25	2	2	2	1.5	0.25	0.25
Gagging	0.25	2	2	2	-	-	-
Regurgitation	0.25	2	2	2	-	-	-
Dyspnea	24	24	24	24	24	24	24
Death	2	2	1	2.3	1.5	1.5	0.5

a. Owens and Ward 1974.
b. Guinea pig.

The mortality rates and LCt_{50}'s are presented in Table 5. Of all the animals (all species) that died, 82, 97, and 100 percent died within 24 hr, 14 days, and 21 days, respectively, after exposure. Death was attributed to suffocation due to the large amounts of particulate matter blocking the respiratory passages. The LCt_{50} for all species combined was 647,470 mg·min/m^3, with a 95 percent confidence limit of 568,611 to 737,265 mg·min/m^3. The LCt_{50} for all rodents combined (rats and guinea pigs) was 481,976 mg·min/m^3, and that for all nonrodents (monkeys, dogs, and rabbits) was 903,586 mg·min/m^3. Thus, rodents were more sensitive to acute effects of inhaling combusted red smoke. Goats and swine were the most resistant species, because the highest Ct's, >1,000,000 mg·min/m^3, caused only 50 percent and 33 percent mortality, respectively, whereas in the remaining species this dose caused 100 percent mortality.

Owens and Ward (1974) also evaluated the effects of inhaling violet smoke in the same species. Violet smoke grenades contain 42 parts (by weight) violet smoke mix, which is composed of 80 percent 1,4-diamino-2,3-dihydroanthraquinone and 20% Disperse Red 9. The concentrations ranged from 1,344 to 10,595 mg/m^3, the exposure time ranged from 8 to 142 min, and the Ct's ranged from 11,626 to 858,262 mg·min/m^3.

Toxic signs included dyspnea, gagging, vomiting, wheezing, generalized weakness, lethargy, ataxia, and prostration. The absence of colored substances in urine, even in the presence of gastrointestinal symptoms, indicated that the dyes were not absorbed and excreted via the urinary tract.

The mortality rate and LCt_{50}'s showed that combusted violet smoke was more lethal than combusted red smoke. The LCt_{50}'s in decreasing order of sensitivity were as follows: monkey 39,731 mg·min/m^3, rabbit 114,756 mg·min/m^3, guinea pig 176,448 mg·min/m^3, rat 240,130 mg·min/m^3, dog 349,950 mg·min/m^3, swine 380,753 mg·min/m^3, and goat 399,831 mg·min/m^3.

TABLE 5. ACUTE TOXICITY IN SEVEN ANIMAL SPECIES FOLLOWING INHALATION OF RED SMOKE DISPERSED FROM AN M18 GRENADE[a]

Species	Ct ($mg \cdot min/m^3$)	Conc (mg/m^3)	Exp. time (min)	Mortality[b] (%)	LCt_{50}[c] ($mg \cdot min/m^3$)
Monkey	331,000	2,758	120	0	
	648,640	4,324	150	0	
	726,850	4,846	150	0	815,013
	788,158	5,254	150	0	
	814,940	3,396	240	50	
	1,029,425	7,625	135	100	
Dog	179,460	17,946	10	0	
	255,084	9,110	28	0	
	331,000	2,758	120	33	
	453,980	3,775	120	83	455,805
	648,640	4,324	150	83	
	726,850	4,846	150	67	
	1,112,795	8,243	135	100	
Goat	331,000	2,758	120	0	
	648,640	4,324	150	0	d
	814,940	3,396	240	0	
	1,112,940	8,243	135	50	
Swine	331,000	2,758	120	0	
	751,890	5,013	150	0	
	814,940	3,396	240	17	d
	1,112,795	8,243	135	33	
Rabbit	433,560	3,613	120	0	
	588,615	6,925	85	67	
	751,890	5,013	150	83	592,306
	788,158	5,254	150	83	
	814,940	3,396	240	100	
Rat	255,084	9,110	28	0	
	433,560	3,613	120	5	
	588,615	6,925	85	85	586,934
	751,890	5,013	150	45	
	788,158	5,254	150	95	
	1,029,425	7,625	135	100	

TABLE 5. continued

Species	Ct ($mg \cdot min/m^3$)	Conc (mg/m^3)	Exp. time (min)	Mortality[b] (%)	LCt_{50}[c] ($mg \cdot min/m^3$)
Guinea pig	179,460	17,946	10	0	
	194,200	6,473	30	45	
	255,084	9,110	28	5	
	433,560	3,613	120	50	
	588,615	6,925	85	95	352,667
	751,890	5,013	150	90	
	1,029,425	7,625	135	100	

a. Owens and Ward 1974.
b. Total number of animals: monkeys, dogs, goats, swine, and rabbits = 6/dose; rats and guinea pigs = 20/dose.
c. Lethal concentration x time causing 50% mortality; represents the Bliss statistical analysis of dose-response data.
d. No analysis possible.

The LCt_{50} for each species was much lower than the corresponding one calculated for combusted red smoke. Monkeys, the most resistant species (LCt_{50}'s not calculated for goats and swine) when exposed to red smoke, were the most sensitive when exposed to violet smoke. Because red smoke was less toxic than violet smoke, and Disperse Red 9 was only 20 percent (by weight) of the violet smoke mixture, it is unlikely that the toxicity of violet smoke was caused by Disperse Red 9.

4.2.2 Human Data

Very few data on the effects of acute exposure to Disperse Red 9 were available. Acute dermal contact with Disperse Red 9 causes irritation and sensitization of the skin in humans (Tatyrek 1965, Dacre et al. 1979). The dermal reaction may be delayed for 2 weeks, with individuals becoming more sensitive to subsequent contact (Tatyrek 1965).

Parent (1964) reported that Disperse Red 9 is only slightly toxic by oral and inhalation exposure. Because the toxicity was slight and the effects were reversible even in the absence of medical treatment, a toxicity rating of "1" was assigned to Disperse Red 9.

4.3 SUBCHRONIC AND CHRONIC TOXICITY

4.3.1 Animal Data

In a mammary tumor bioassay, a total of 5000 mg/kg of Disperse Red 9, in ten equal doses over a period of 30 days, was administered by gastric intubation to 19 Sprague-Dawley rats (Griswold et al. 1968). At the end of the treatment period, very little toxicity was noted; one animal died during treatment and four died during the 9-month observation period. In 140 controls treated with sesame oil only, six animals died during treatment and seven died during the observation period. Thus, according to Griswold et al. (1968), Disperse Red 9 exhibited little toxicity.

Another report, by Marrs et al. (1984), described the toxic effects of chronic inhalation exposure to a smoke mix containing 16 percent Disperse Red 9, 13 percent Solvent Yellow 33, and 19 percent Solvent Green 3. Three animal species, 400 Porton-strain SPF female mice, 200 Porton Wistar-derived female rats, and 200 Dunkin-Hartley female guinea pigs, were exposed to the combusted colored smoke for 1 hr/day, 5 days/week for 20 weeks (100 exposures), at concentrations of 105.8 mg/m^3 (low dose), 309.6 mg/m^3 (medium dose), and 1012.4 mg/m^3 (high dose, mice, rats) or 1161.1 mg/m^3 (high dose, guinea pigs). Starting with the initiation of exposure, the animals were observed for 71 weeks for toxicity effects and then sacrificed for histopathological evaluation.

Because the animals were exposed to a mixture of dyes, the effects could not be attributed to Disperse Red 9 alone. During the treatment period, the mortality rates were low in all groups, with the exception of the guinea pigs exposed to the high dose. After 16 exposures, treatment of guinea pigs was discontinued because of a high intercurrent mortality during exposure, which was 18 percent after 4 weeks. Dose-related trends in mortality rates were not significant in mice, rats, and low- and medium-dose guinea pigs (F-test). In high-dose guinea pigs, the mortality rate at 71 weeks was 28 percent, compared with 12 percent in the control group; a dose-related trend in the mortality rate was not significant (chi-square test).

Mean body weights of treated and control groups, related to age, were significantly different ($P < 0.005$) during the treatment period (Kolmogorov-Smirnov test). Body weights at death were significantly different only in rats exposed to medium and high doses. Guinea pigs exposed to the high dose lost weight rapidly during the exposure, but body weights stabilized after treatment was terminated.

Organ weights were not affected by treatment, with the exception of lung weights in mice and rats. The lungs in mice exposed to the high dose weighed more than those in mice exposed to medium and low doses ($P < 0.05$), and the lungs in rats exposed to the high dose weighed more than those in control rats ($P < 0.001$).

Histopathological evaluation of all animals dying prior to or surviving until termination revealed changes related almost exclusively to the respiratory tract. In mice sacrificed at 40 weeks, significant dose-related trends for severe chronic pneumonia ($P < 0.001$), bronchiectasis ($P < 0.001$), and alveolitis ($P < 0.05$) were revealed. These changes were attributed to nonspecific damage caused by inhaling particulate matter, and not to specific toxic effects of colored smoke. At 71 weeks, significant dose-related trends were observed for the presence of alveolar macrophages ($P < 0.001$), combined incidence of mild and severe chronic pneumonia ($P < 0.05$), and fatty livers ($P < 0.05$).

In rats evaluated at 71 weeks, significant trends were observed for the presence of submucosal lymphocytes in the larynx ($P < 0.05$) and trachea ($P < 0.01$), perivascular lymphocyte aggregates ($P < 0.001$), alveolitis ($P < 0.05$), and mild and severe foreign-body reaction characterized by the presence of alveoli packed with macrophages ($P < 0.001$). According to Marrs et al. (1984), the foreign-body reaction often caused complete obliteration of alveolar spaces, which should have led to a loss of respiratory capacity and a high mortality rate. The mortality rate, however, was not affected.

In guinea pigs, a significant increase in the incidence of severe alveolitis was observed in the low- and medium-dose groups ($P < 0.05$), but not in the high-dose group, which received only 16 exposures.

The incidence of hyperplastic and neoplastic lesions will be discussed in Section 4.6.

4.3.2 Human Data

No data were found.

4.4 GENOTOXICITY

Brown and Brown (1976) tested 90 anthraquinone derivatives, including Disperse Red 9, 1-aminoanthraquinone, a urinary metabolite (Martin et al. 1983) and combustion product of Disperse Red 9 and 2-aminoanthraquinone, another combustion product (Rubin, Buchanan, and Moneyhun 1982), for mutagenic potential in Salmonella typhimurium strains TA1535, TA100, TA1537, TA1538, TA1978, and TA98. Disperse Red 9, in doses of 100 to 200 μg/disk, was not mutagenic in preliminary spot tests in the presence or absence of metabolic activation by the microsomal fraction (S9) from Aroclor 1254 induced rat liver. According to Brown and Brown (1976), the metabolites and combustion products, 1-aminoanthraquinone and 2-amino-anthrahraquinone, were mutagenic in the preliminary spot and plate tests. Purification and analysis of these compounds by thin-layer chromatography showed that they contained sufficient quantities of

1,2-diaminoanthraquinone (a strong mutagen in the presence and absence of S9) to elicit a positive response.

Brusick and Matheson (1978) carried out several different in vitro and in vivo mutagenicity tests with Disperse Red 9. They reported that Disperse Red 9 in doses ranging from 0.1 to 1,000 μg/plate was not mutagenic in five strains of Salmonella typhimurium with or without metabolic activation by S9, thus confirming the results of Brown and Brown (1976). They also found that the same doses were not mutagenic in yeast Saccharomyces cerevesiae strain D4.

Disperse Red 9 was tested in Fischer mouse lymphoma cells ($L5178Y/TK^{+/-}$) in doses of 0.10, 0.20, 0.25, 0.38, and 0.50 mg/mL in the absence of S9 and 0.05, 0.20, 0.25, 0.38, and 0.50 mg/mL in the presence of S9 (Brusick and Matheson 1978). Forward mutations, $TK^{-/-}$, were selected by growing the cells in bromodeoxyuridine (Budr). The mutation index for each dose level was compared with positive controls, negative controls, and other dose levels.

The results of these tests are presented in Table 6. Disperse Red 9 was very cytotoxic to mouse lymphoma cells, especially in the presence of S9. The criteria for a positive mutagenic response established by Brusick and Matheson (1978) were: (1) a 2.5-fold increase in mutant frequency at high dose levels compared with concurrent solvent control, (2) a dose-response relationship observed over three of the four dose levels employed, and (3) a solvent control value within the normal range of the spontaneous background for the TK locus. In addition, Turner et al. (1984) suggested that cell survival, relative to solvent control, should be 10 percent or greater. In the absence of S9, the relative growth at the two highest concentrations was less than 10 percent, and two of the three lowest concentrations (0.20 and 0.25 mg/mL) were so close as to represent duplicates. Nevertheless, the data indicated that Disperse Red 9, in the absence of S9, was mutagenic in mouse lymphoma cells. In the presence of S9, the percent relative growth was very low for the four highest doses. At the lowest dose, however, the relative growth was 20.8 percent and the mutation frequency was six times that of the solvent control, indicating that Disperse Red 9 was also mutagenic in the presence of S9.

The third test, for unscheduled DNA synthesis (excision repair) as measured by incorporation of [^{3}H]thymidine, was performed in human diploid WI-38 cells derived from embryonic lungs (Brusick and Matheson 1978). Confluent, nonproliferating cells were exposed to Disperse Red 9 in doses of 50, 100, 500, and 1000 μg/mL along with [^{3}H]thymidine in the presence and absence of metabolic activation by S9.

In the absence of S9, the specific activity of the DNA (dpm/μg DNA), was lower than that of the solvent control at each dose tested, whereas in the presence of S9 the specific activity of DNA was 1.8 and 2.8 times that of the solvent control at doses of 50 and 100 μg/mL, respectively, but the

TABLE 6. SUMMARY OF MOUSE LYMPHOMA MUTAGENICITY ASSAY[a]

Test System[b]	Rel. Susp. Growth[c] (% of Control)	% Relative Growth[d]	Mutant Frequency[e]
Nonactivation			
DMSO control	100.0	100.0	12.5
Negative control	76.4	83.9	11.8
EMS (0.5 μL/L)	39.3	18.8	295.9
Disperse Red 9			
0.10 mg/mL	36.6	34.3	27.2
0.20 mg/mL	37.9	20.1	50.4
0.25 mg/mL	15.1	10.8	32.4
0.38 mg/mL	10.7	5.1	52.1
0.50 mg/mL	4.1	1.2	48.6
S9 Activation			
DMSO control	100.0	100.0	10.1
Negative control	148.9	127.6	11.4
DMN (0.5 μL/L)	30.8	7.2	245.7
Disperse Red 9			
0.05 mg/mL	35.7	20.8	68.8
0.20 mg/mL	15.8	3.4	128.6
0.25 mg/mL	14.1	2.2	110.6
0.38 mg/mL	15.6	2.3	160.5
0.50 mg/mL	12.4	2.7	64.1

a. Adapted from Brusick and Matheson 1978.
b. DMSO = dimethylsulfoxide, EMS = ethylmethanesulfonate, DMN = dimethylnitrosamine.
c. Rel. Susp. Growth = Relative Suspension Growth.
d. (Relative suspension growth x relative cloning efficiency)/100
e. (Mutant clones/viable clones) x 10^{-6}.

specific activity at higher doses was lower than that of controls. The specific activity of DNA in the solvent control in the presence of S9 was much lower than that of the solvent control in the absence of S9. Also, the amount of DNA on plates exposed to the two highest doses was much higher than the amount on the plates exposed to the lowest doses, indicating that more cells were present on the plates at the time of exposure, which may have affected the uptake of [^{3}H]thymidine.

In a repeat test, the amount of DNA on all plates exposed to Disperse Red 9 and solvent was similar. As in the first test, stimulation of incorporation of [^{3}H]thymidine was not observed at any dose in the absence of S9; but in the presence of S9, the 50 μg/mL dose stimulated incorporation by 197 percent. Inhibition of incorporation of [^{3}H]thymidine was observed at all other doses in the presence and absence of S9. Therefore, the data showed that Disperse Red 9, in the presence of S9, was active in

the unscheduled DNA synthesis assay. Brusick and Matheson (1978) also stated that Disperse Red 9 was toxic to WI-38 cells, but the data did not indicate a toxic effect.

Brusick and Matheson (1978) performed a dominant lethal assay in mice and rats according to established protocol (Bateman 1984, USEPA 1985). Disperse Red 9 was administered in the diet at doses of 0.2, 0.67, and 2.0 g/kg to 10 random bred male mice for 5 consecutive days. The treated males were then mated with virgin females. The females were killed 14 days after the midpoint of mating, and the number of dead and living implants were counted for each female. Statistical analyses applied to the data included chi-square test, Armitage's trend for linearity of arithmetic or logarithmic dose, Student's t-test, and regression analysis. The analyses did not show dose-related trends for any of the parameters examined in mice. Therefore, Brusick and Matheson (1978) considered Disperse Red 9 to be negative in the assay.

A similar test was performed in ten random bred rats administered Disperse Red 9 by gavage for 5 consecutive days. In the week 4 mating, the number of dead implants per pregnant female, the proportion of females with one or more dead implants, the proportion of females with two or more dead implants, and the number of dead implants per total implants were statistically higher in animals administered the high dose (2.0 g/kg), than in solvent controls. Nevertheless, Brusick and Matheson (1978) considered Disperse Red 9 to be negative in this assay. They suggested that the statistical differences were due to unusually low values in solvent controls, but it should be noted that the values in test animals were also much higher than those reported for historical controls. The values in test animals, however, were not statistically compared with those in historical controls.

4.5 DEVELOPMENTAL/REPRODUCTIVE TOXICITY

Data on the developmental and reproductive toxicity of Disperse Red 9 were not found.

4.6 ONCOGENICITY

In a rat mammary tumor bioassay, Griswold et al. (1968) administered 5000 mg of Disperse Red 9 to each of 19 female Sprague-Dawley rats by gastric intubation in ten equal doses at intervals of 3 days. The surviving animals were killed 9 months after initiating treatment. In the 18 animals autopsied, only one hyperplastic lesion was observed in the mammary gland in one animal. One unusual neoplastic lesion, a tubular adenocarcinoma of the kidney, was also observed in one animal. Of the 132 control animals examined, three carcinomas, one fibroadenoma, and five hyperplastic lesions were observed in the mammary gland of five animals. Neoplastic lesions at other sites were not found in control animals.

According to Griswold et al. (1968), Disperse Red 9 was not carcinogenic under the conditions of this test. The unusual neoplastic lesion in the kidney suggests that a similar test should be repeated using the guidelines established by the USEPA (1985).

Slaga et al. (1986) used the SENCAR Mouse Bioassay System (two-stage mouse skin carcinogenesis system) to study the tumorigenicity of Disperse Red 9 in the skin. Disperse Red 9 in 0.2 mL of acetone was applied to the shaved backs of 40 SENCAR mice (20 of each sex). To test Disperse Red 9 as a complete carcinogen, the following treatment groups were included: (1) application of 2 mg of the dye followed 7 days later by twice weekly applications of 1 mg for 30 weeks, (2) application of 1 mg of the dye followed 7 days later by once weekly applications of 1 mg for 30 weeks, and (3) application of 0.1 mg of the dye followed 7 days later by once weekly applications of 0.1 mg for 30 weeks. A positive control group received 2.52 μg of 7,12-dimethylbenz(a)anthracene (DMBA) followed by once weekly applications of the same amount for 30 weeks. No papillomas or carcinomas were observed at the end of the treatment period in the animals receiving the dye.

Three groups of mice were also treated with violet smoke dye composed of 20 percent Disperse Red 9 and 80 percent 1,4-diamino-2,3-dihydroanthraquinone. The protocol was identical to that of animals treated with Disperse Red 9. No papillomas or carcinomas were observed at the end of the treatment period in the animals receiving the dye.

The SENCAR Mouse Skin Bioassay System was also used to test Disperse Red 9 and violet smoke dye for tumor initiating activity (Slaga et al. 1986). Groups of 40 SENCAR mice (20 of each sex) were given a single topical application of 2 mg, 1 mg, or 0.1 mg of each dye in 0.2 ml of acetone (initiation) followed 7 days later by twice weekly applications of 2 μg of a known mouse skin tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA) for 30 weeks (promotion). Positive controls received a single application of 2.52 μg of DMBA followed by twice weekly applications of TPA, and negative controls received a single application of acetone followed by TPA. The results are presented in Table 7.

Although animals treated with the dyes developed papillomas, the incidence and the papillomas per mouse were not statistically different from those of mice initiated with acetone and promoted with TPA.

Marrs et al. (1984) exposed mice, rats, and guinea pigs to a smoke mixture containing 16 percent Disperse Red 9, 13 percent Solvent Yellow 33, and 19 percent Solvent Green 3 for 1 hr/day, 5 days/week for 20 weeks at concentrations of 105.8 mg/m^3, 309.6 mg/m^3, and 1012.4 mg/m^3 or 1161.1 mg/m^3. Further details of this experiment were presented in Section 4.3.

Seventy-one weeks after initiating treatment, histopathological evaluation revealed three lesions in medium-dose and two lesions in

TABLE 7. TUMOR INITIATING ACTIVITY OF DISPERSE RED 9 AND VIOLET SMOKE DYE IN SENCAR MICE PROMOTED WITH TPA[a]

Compound	Dose	Papillomas per Mouse	% Mice with Papillomas	Carcinomas per Mouse
Disperse Red 9	2 mg	0.15	12.5	0
	1 mg	0.0	0	0
	0.1 mg	0.1	7.5	0
Violet Dye	2 mg	0.05	5.2	0
	1 mg	0.075	7.5	0
	0.1 mg	0.025	2.6	0
DMBA	2.52 μg	10.8	100	0.09
Acetone + TPA	2 μg	0.025	2.6	0

a. Adapted from Slaga et al. 1986.

high-dose mice classified as hepatoma a, and one lesion classified as hepatoma b in low-dose mice (no significant dose-related trend). One adenocarcinoma of the breast was observed in the low- and medium-dose groups, but the incidence did not show a significant dose-related trend.

In rats killed 71 weeks after initiating exposure, one adenocarcinoma and one squamous cell carcinoma of the lungs were observed, but a significant dose-related trend was not observed. In addition, two hemangiomas in the adrenal gland in the high-dose group ($P < 0.05$), one biliary hyperplastic lesion in the medium-dose and four in the high-dose groups ($P < 0.01$), and three adenocarcinomas of the breast in the high-dose group were significant for dose-related trends. The incidence of neoplastic lesions in exposed guinea pigs was not significantly different from that in controls.

4.7 SUMMARY

Studies in pharmacokinetics conducted in sheep showed that Disperse Red 9 administered orally is absorbed from the gastrointestinal tract, metabolized by N-demethylation, 2-, or 4-hydroxylation, and glucuronide conjugation, and eliminated by urinary excretion. Colored metabolites of Disperse Red 9 recovered in urine are glucuronide-conjugated and unconjugated 1-aminoanthraquinone, 2-hydroxy-1-aminoanthraquinone, and 4-hydroxy-1-aminoanthraquinone, in addition to conjugated and unconjugated Disperse Red 9. The predominant metabolite, 2-hydroxy-1-aminoanthraquinone (unconjugated plus conjugated), constitutes more than 70 percent of all metabolites recovered in urine. Colored metabolites were also detected in urine of dogs administered 4 or 8 g/kg of Disperse Red 9

orally and in other laboratory animals exposed to combusted red smoke by inhalation.

In sheep, very small quantities of both conjugated and unconjugated metabolites accumulated in liver, kidney, and bile, whereas only unconjugated metabolites accumulated in milk. Unmetabolized Disperse Red 9 was eliminated in feces.

Because colored metabolites did not appear in urine, it was concluded that Disperse Red 9 applied topically is not absorbed through the skin. Inhaled Disperse Red 9 is not retained in the respiratory tract, but animals exposed to Disperse Red 9 by inhalation could swallow the dye, which could then be absorbed from the gastrointestinal tract, or the dye could be absorbed directly from the respiratory tract.

Disperse Red 9 administered orally causes no effect or only mild toxic effects in laboratory animals. Changes in body weight, blood chemistry, hematology, and histopathology are not observed in dogs administered up to 8 g/kg. Disperse Red 9 in doses of 0.2 to 2.0 g/kg/day administered for 5 days to mice and rats or 500 mg/day administered to rats for 3 days is only mildly toxic. Disperse Red 9 applied to the skin of rabbits is neither toxic nor irritating, and it causes only temporary discomfort and conjunctival erythema when applied to the eyes.

In laboratory animals, immediate effects of inhaling combusted red smoke or Disperse Red 9 are eye irritation, nasal discharge, gasping, lethargy, salivation, gagging, regurgitation, dyspnea, and death, depending on the species and dose. Systemic toxicity, probably due to direct absorption of the dye from the lungs or to absorption of swallowed smoke particles from the gastrointestinal tract, is indicated by a decrease in weight gain.

For inhalation exposure to Disperse Red 9, the LCt_{50} for all species combined (rats, guinea pigs, rabbits, dogs, and monkeys) is 647,470 $mg \cdot min/m^3$; for rodents the LCt_{50} is 481,976 $mg \cdot min/m^3$, and for nonrodents the LCt_{50} is 903,586 $mg \cdot min/m^3$. The decreasing order of sensitivity as indicated by LCt_{50}'s is guinea pigs > dogs > rats > rabbits > monkeys. Although the mortality data were insufficient for calculating LCt_{50}'s, goats and swine are more resistant than monkeys.

Inhalation of combusted violet smoke mix (80 percent 1,4-diamino-2,3-dihydroxyanthraquinone and 20 percent Disperse Red 9) causes more severe toxic effects than inhalation of combusted red smoke. Because the LCt_{50}'s are much lower for inhaling violet smoke than for inhaling red smoke, and because the amount of Disperse Red 9 in violet smoke is only 20 percent of that in red smoke, it is unlikely that the toxic effects of violet smoke are due to Disperse Red 9.

In humans, dermal contact with Disperse Red 9 causes irritation and delayed sensitivity of the skin. It is only mildly toxic by acute oral and inhalation exposure; therefore, the toxicity rating assigned to Disperse Red 9 is "1."

In laboratory animals, chronic exposure to Disperse Red 9 by the oral route causes only mild toxic effects. Chronic inhalation exposure to a smoke mixture containing Disperse Red 9, Solvent Yellow 33, and Solvent Green 3 causes nonspecific damage to the respiratory tract in mice, rats, and guinea pigs. Because the animals were exposed to other dyes in addition to Disperse Red 9, the effects observed in this study could not be attributed to one specific dye.

The results of several genotoxicity tests revealed that Disperse Red 9 is nonmutagenic in five strains of Salmonella typhimurium in both the presence and absence of S9. In doses ranging from 0.05 to 0.50 mg/mL, Disperse Red 9 is mutagenic in mouse lymphoma cells in the presence and absence of S9, and positive in the unscheduled DNA synthesis assay in human diploid WI-38 cells only in the presence of S9. Disperse Red 9 is reported to be negative in the dominant lethal assay in both mice and rats. Nevertheless, in rats, the data related to the number of dead implants per pregnant female or per total implants from the week 4 mating showed that the values of these parameters are statistically higher in animals treated with 2.0 g/kg of Disperse Red 9 than in solvent controls, suggesting that Disperse Red 9 may cause dominant lethality, but not in stem cells.

One study showed that Disperse Red 9 applied topically is not active as a complete carcinogen in the SENCAR Mouse Bioassay System (two-stage mouse skin carcinogenesis system). A few papillomas appeared when Disperse Red 9 was tested as a tumor initiator, but the incidence was not significantly greater than that in mice initiated with acetone and promoted with TPA. Significant increases in the incidences of hemangiomas in the adrenal gland, biliary hyperplasia, and adenocarcinomas of the breast were observed in rats exposed by inhalation to Disperse Red 9 in a mixture with Solvent Green 3 and Solvent Yellow 33; carcinogenicity, however, could not be attributed to Disperse Red 9 alone. In another study, one unusual neoplastic lesion in the kidney was observed in rats exposed to Disperse Red 9 by gastric intubation.

Available data on the toxicity of Disperse Red 9 in humans are scarce. No data are available on the pharmacokinetics, subchronic and chronic toxicity, genotoxicity, developmental and reproductive toxicity, and genotoxicity.

5. CRITERION FORMULATION

5.1 EXISTING GUIDELINES AND STANDARDS

Standards for occupational exposure or exposure of the general population specifically to Disperse Red 9 do not exist. During the production of colored smoke grenades from raw materials, workers may be exposed to particulates of the dyes (dust). The OSHA standards (8-hr time-weighted average) for the levels of inert or nuisance dust in the occupational environment are 15 mg/m^3 of total dust or 5 mg/m^3 of respirable dust (USOSHA 1985). The threshold limit value for inert or nuisance dust is 10 mg/m^3 of total dust or 5 mg/m^3 of respirable dust (ACGIH 1985, ILO 1980). The Federal Ambient Air Quality Standard for particulate matter is 75 $\mu g/m^3$ annual geometric mean and 260 $\mu g/m^3$ for a maximum 24-hr concentration not to be exceeded more than once per year (USEPA 1981, as reported in Cichowicz and Wentsel 1983).

The Surgeon General has established interim guidelines for the disposal of colored smokes. There should be no open burning and personnel should not be exposed to dye components at levels above 0.2 mg/m^3 (8-hr time-weighted average) (Cichowicz and Wentsel 1983).

5.2 OCCUPATIONAL EXPOSURE

Occupational exposure standards specifically for Disperse Red 9 do not exist. Data on the levels of exposure in the occupational environment, or the levels of exposure during deployment of munitions containing Disperse Red 9, were not available. According to Garcia et al. (1982), the levels of dust in the colored smoke grenade production facility at the Pine Bluff Arsenal, Pine Bluff, Arkansas, exceeded the limits established by OSHA.

5.3 PREVIOUSLY CALCULATED CRITERIA

Aquatic or human health criteria have not been calculated for Disperse Red 9.

5.4 AQUATIC CRITERIA

A brief description of the methodology proposed by the USEPA for the estimation of water quality criteria for the protection of aquatic life and its uses is presented in Appendix A. The aquatic criteria consist of two values, a Criterion Maximum Concentration (CMC) and a Criterion

Continuous Concentration (CCC) (Stephan et al. 1985). The CMC is equal to one-half the Final Acute Value (FAV), while the CCC is equal to the lowest of the Final Chronic Value, the Final Plant Value, or the Final Residue Value.

No data are available in the literature concerning the toxicity of Disperse Red 9 to aquatic organisms; consequently, neither a CMC nor a CCC can be calculated.

5.5 HUMAN HEALTH CRITERIA

Anthraquinones are a class of compounds that have carcinogenic potential. Of the three studies on the carcinogenicity of Disperse Red 9, one demonstrated that the dye was not carcinogenic in the skin (Slaga et al. 1986) and the other two were inconclusive (Griswold et al. 1968, Marrs et al. 1984). No data on the carcinogenicity of Disperse Red 9 in humans were available. Therefore, a criterion based on carcinogenicity (nonthreshold chronic toxicity) cannot be calculated.

Threshold chronic toxicity data in humans were not available, and chronic toxicity data in laboratory animals were insufficient to calculate a criterion. In the long-term study available (20-week treatment time), the animals were exposed by inhalation to Solvent Green 3 and Solvent Yellow 33, in addition to Disperse Red 9 (Marrs et al. 1984). Because the toxic effects could not be attributed to Disperse Red 9 alone, the data were insufficient for calculating a criterion.

The USEPA guidelines for deriving a water quality criterion for the protection of human health (USEPA 1980) are summarized in Appendix B. Based on these guidelines, additional experimentation is required in order to provide the necessary data to calculate a criterion.

5.6 RESEARCH RECOMMENDATIONS

In order to meet the requirements established by the USEPA for deriving water quality criteria, the following research studies are recommended to fill gaps in the existing data.

1. In order to calculate an FAV, acute toxicity tests following USEPA procedures must be performed for at least 8 different families of aquatic organisms, including: a) member of family Salmonidae in class Osteichthyes; b) member of second family in class Osteichthyes, preferably an important warmwater species; c) member of family in phylum Chordata; d) planktonic crustacean; e) benthic crustacean; f) member of class Insecta; g) member in phylum other than Arthropoda or Chordata; and h) member of family in order of insect or phylum not represented.

2. Conduct chronic flow-through tests using measured concentrations for an invertebrate species, a fish species, and a sensitive freshwater species in order to calculate a Final Chronic Value.

3. Conduct acute flow-through tests using measured concentrations for the three aquatic species for which chronic tests are being performed in order to calculate acute-chronic ratios.

4. Conduct a conclusive toxicity test with an alga or aquatic vascular plant using measured concentrations and a biologically important end point in order to calculate a Final Plant Value.

5. Conduct a definitive steady-state or 28-day bioaccumulation study. Determine a maximum permissible tissue concentration by conducting a chronic wildlife feeding study or a long-term wildlife field study. These data will provide information to calculate a Final Residue Value.

6. Very few definitive tests on the toxicity of Disperse Red 9 have been performed in rodent species. In order to fill data gaps, the following toxicity studies should be performed, in either rats or mice, according to USEPA Toxic Substances Control Act Test Guidelines (USEPA 1985): acute oral toxicity tests, chronic oral toxicity/oncogenicity with preliminary subchronic dose range tests, chronic inhalation with subchronic dose range tests, and oral and inhalation reproductive/develop mental toxicity tests. The results of oral toxicity tests are important for derivation of a water quality criterion for the protection of human health. Inhalation tests are recommended because inhalation is possibly the major route of exposure for workers and military personnel.

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7. GLOSSARY

ACGIH	American Conference of Governmental Industrial Hygienists
Budr	Bromodeoxyuridine
CCC	Criterion Continuous Concentration
CMC	Criterion Maximum Concentration
Ct	Concentration x time
DMBA	7,12-Dimethylbenz(a)anthracene
dpm	Disintegration per minute
FAV	Final Acute Value
LCt_{50}	Lethal concentration x time causing 50% mortality
OSHA	Occupational Safety and Health Administration
NMR	Nuclear Magnetic Resonance
TPA	12-O-Tetradecanoylphorbol-13-acetate
USEPA	United States Environmental Protection Agency
USITC	United States International Trade Commission

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APPENDIX A:

SUMMARY OF USEPA METHODOLOGY FOR DERIVING NUMERICAL WATER QUALITY CRITERIA FOR THE PROTECTION OF AQUATIC ORGANISMS AND THEIR USES

The following summary is a condensed version of the 1985 final U.S. Environmental Protection Agency (USEPA) guidelines for calculating water quality criteria to protect aquatic life with emphasis on the specific regulatory needs of the U.S. Army (e.g., discussion of saltwater aspects of the criteria calculation are not included). The guidelines are the most recent document outlining the required procedures and were written by the following researchers from the USEPA's regional research laboratories: C.E. Stephan, D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. For greater detail on individual points consult Stephan et al. (1985).

1. INTRODUCTION

The Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses describe an objective, internally consistent, and appropriate way of estimating national criteria. Because aquatic life can tolerate some stress and occasional adverse effects, protection of all species at all times was not deemed necessary. If acceptable data are available for a large number of appropriate taxa from a variety of taxonomic and functional groups, a reasonable level of protection should be provided if all except a small fraction are protected, unless a commercially, recreationally, or socially important species is very sensitive. The small fraction is set at 0.05, because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is appropriate.

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally, and socially important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. These national guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations.

Numerical aquatic life criteria derived using these national guidelines are expressed as two numbers, so that the criteria can more accurately reflect toxicological and practical realities. The combination of a maximum concentration and a continuous concentration is designed to provide adequate protection of aquatic life and its uses from acute and chronic toxicity to animals, toxicity to plants, and bioaccumulation by

aquatic organisms without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these guidelines should be useful for developing water quality standards, mixing zone standards, and effluent standards. Development of such standards may have to consider additional factors such as social, legal, economic, and additional biological data. It may be desirable to derive site-specific criteria from these national criteria to reflect local conditions (USEPA 1982). The two factors that may cause the most difference between the national and site-specific criteria are the species that will be exposed and the characteristics of the water.

Criteria should provide reasonable and adequate protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a criterion be the best estimate obtainable using available data; it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Thus, these guidelines require that certain data be available if a criterion is to be derived. If all the required data are not available, usually a criterion should not be derived: however, availability of all required data does not ensure that a criterion can be derived. The amount of guidance in these national guidelines is significant, but much of it is necessarily qualitative rather than quantitative: much judgement will be required to derive a water quality criterion for aquatic life. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these guidelines and should be consistent with the spirit of these guidelines - which is to make best use of all available data to derive the most appropriate criterion.

2. DEFINITION OF MATERIAL OF CONCERN

1. Each separate chemical that does not ionize significantly in most natural bodies of water should be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.

2. For chemicals that do ionize significantly, all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different nonionizable covalently bonded organometallic compound should usually be considered a separate material.

3. Definition of the material should include an operational analytical component. It is also necessary to reference or describe analytical methods that the term is intended to denote. Primary requirements of the operational analytical component is that it be appropriate for use on samples of receiving water, that it be compatible with toxicity and

bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection of aquatic life and its uses.

NOTE: Analytical chemistry of the material may have to be considered when defining the material or when judging acceptability of some toxicity tests, but a criterion should not be based on sensitivity of an analytical method. When aquatic organisms are more sensitive than analytical techniques, the proper solution is to develop better analytical methods, not to underprotect aquatic life.

3. COLLECTION OF DATA

1. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants; (b) FDA action levels (FDA Guidelines Manual); and (c) chronic feeding studies and long-term field studies with wildlife that regularly consume aquatic organisms.

2. All data used should be available in typed, dated, and signed hardcopy with enough supporting information to indicate that acceptable test procedures were used and the results should be reliable.

3. Questionable data, whether published or not, should not be used.

4. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the test material should not be used.

5. For some highly volatile, hydrolyzable, or degradable materials it may be appropriate to only use results of flow-through tests in which concentrations of test material in test solutions were measured using acceptable analytical methods.

6. Do not use data obtained using brine shrimp, species that do not have reproducing wild populations in North America, or organisms that were previously exposed to significant concentrations of the test material or other contaminants.

4. REQUIRED DATA

1. Results of acceptable acute tests (see Section 5) with freshwater animals in at least eight different families such that all of the following are included:

   a. the family Salmonidae in the class Osteichthyes,

b. a second family (preferably an important warmwater species) in the class Osteichthyes (e.g., bluegill, fathead minnow, or channel catfish);

c. a third family in the phylum Chordata (e.g, fish or amphibian);

d. a planktonic crustacean (e.g, cladoceran or copepod);

e. a benthic crustacean (e.g, ostracod, isopod, or amphipod);

f. an insect (e.g., mayfly, midge, stonefly);

g. a family in a phylum other than Arthropoda or Chordata (e.g, Annelida or Mollusca); and

h. a family in any order of insect or any phylum not represented.

2. Acute-chronic ratios (see Section 7) for species of aquatic animals in at least three different families provided that of the three species at least (a) one is a fish, (b) one is an invertebrate, and (c) one is a sensitive freshwater species.

3. Results of at least one acceptable test with a freshwater alga or a chronic test with a freshwater vascular plant (see Section 9). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should be available.

4. At least one acceptable bioconcentration factor determined with an appropriate aquatic species, if a maximum permissible tissue concentration is available (see Section 10).

If all required data are available, a numerical criterion can usually be derived, except in special cases. For example, if a criterion is to be related to a water quality characteristic (see Sections 6 and 8), more data will be necessary. Similarly, if all required data are not available, a numerical criterion should not be derived except in special cases. For example, even if acute and chronic data are not available, it may be possible to derive a criterion if the data clearly indicate that the Final Residue Value would be much lower than either the Final Chronic Value or the Final Plant Value. Confidence in a criterion usually increases as the amount of data increases. Thus, additional data are usually desirable.

5. FINAL ACUTE VALUE

1. The Final Acute Value (FAV) is an estimate of the concentration of material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acute tests have

been conducted on the material. However, in some cases, if the Species Mean Acute Value (SMAV) of an important species is lower than the calculated FAV, then that SMAV replaces the FAV to protect that important species.

2. Acute toxicity tests should have been conducted using acceptable procedures (e.g., ASTM Standard E 724 or 729).

3. Generally, results of acute tests in which food was added to the test solution should not be used, unless data indicate that food did not affect test results.

4. Results of acute tests conducted in unusual dilution water, e.g., dilution water containing high levels of total organic carbon or particulate matter (higher than 5 mg/L), should not be used, unless a relationship is developed between toxicity and organic carbon or unless data show that organic carbon or particulate matter, etc. do not affect toxicity.

5. Acute values should be based on endpoints which reflect the total adverse impact of the test material on the organisms used in the tests. Therefore, only the following kinds of data on acute toxicity to freshwater aquatic animals should be used:

   a. Tests with daphnids and other cladocerans should be started with organisms < 24 hr old, and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC_{50} based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} is not available from a test, the 48-hr LC_{50} should be used in place of the desired 48-hr EC_{50}. An EC_{50} or LC_{50} of longer than 48 hr can be used provided animals were not fed and control animals were acceptable at the end of the test.

   b. The result of tests with all other aquatic animal species should be the 96-hr EC_{50} value based on percentage of organisms exhibiting loss of equilibrium plus percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} value is not available from a test, the 96-hr LC_{50} should be used in place of the desired EC_{50}.

   c. Tests with single-cell organisms are not considered acute tests, even if the duration was ≤ 96 hr.

   d. If the tests were conducted properly, acute values reported as greater than values and those acute values which are above solubility of the test material are acceptable.

6. If the acute toxicity of the material to aquatic animals has been shown to be related to a water quality characteristic (e.g., total organic carbon) for freshwater species, a Final Acute Equation should be derived based on that characteristic.

7. If the data indicate that one or more life stages are at least a factor of 2 times more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the SMAV, because a species can only be considered protected from acute toxicity if all life stages are protected.

8. Consider the agreement of the data within and between species. Questionable results in comparison with other acute and chronic data for the species and other species in the same genus probably should not be used.

9. For each species for which at least one acute value is available, the SMAV should be calculated as the geometric mean of all flow-through test results in which the concentrations of test material were measured. For a species for which no such result is available, calculate the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial total concentrations of test material.

   NOTE: Data reported by original investigators should not be rounded off, and at least four significant digits should be retained in intermediate calculations.

10. For each genus for which one or more SMAV is available, calculate the Genus Mean Acute Value (GMAV) as the geometric mean of the SMAVs.

11. Order the GMAVs from high to low, and assign ranks (R) to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.

12. Calculate the cumulative probability (P) for each GMAV as R/(N+1).

13. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are <59 GMAVs, these will always be the four lowest GMAVs).

14. Using the selected GMAVs and Ps, calculate

$$s^2 = \frac{\Sigma((\ln GMAV)^2) - ((\Sigma(\ln GMAV))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4)}$$

$$L = (\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P})))/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

15. If for an important species, such as a recreationally or commercially important species, the geometric mean of acute values from flow-through tests in which concentrations of test material were measured is lower than the FAV, then that geometric mean should be used as the FAV.

16. Go to Section 7.

6. FINAL ACUTE EQUATION

1. When enough data show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

2. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of acute toxicity values on values of the water quality characteristic.

3. Decide whether the data for each species is useful, considering the range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used.

4. Individually for each species calculate the geometric mean of the acute values and then divide each of the acute values for a species by the mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.

5. Similarly normalize the values of the water quality characteristic for each species individually.

6. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 2. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

7. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope (V) and its 95 percent confidence limits. If all the normalized data are

actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

8. For each species calculate the geometric mean (W) of the acute toxicity values and the geometric mean (X) of the related values of the water quality characteristic (calculated in 4. and 5. above).

9. For each species calculate the logarithmic intercept (Y) of the SMAV at a selected value (Z) of the water quality characteristic using the equation: $Y = \ln W - V(\ln X - \ln Z)$.

10. For each species calculate the SMAV using: $SMAV = e^{Y}$.

11. Obtain the FAV at Z by using the procedure described in Section 5 (Nos. 10-14).

12. If the SMAV for an important species is lower than the FAV at Z, then that SMAV should be used as the FAV at Z.

13. The Final Acute Equation is written as:

$$FAV = e^{(V[\ln(\text{water quality characteristic})] + \ln A - V[\ln Z])},$$

where V = pooled acute slope and A = FAV at Z. Because V, A, and Z are known, the FAV can be calculated for any selected value of the water quality characteristic.

7. FINAL CHRONIC VALUE

1. Depending on available data, the Final Chronic Value (FCV) might be calculated in the same manner as the FAV or by dividing the FAV by the Final Acute-Chronic Ratio.

   NOTE: Acute-chronic ratios and application factors are ways of relating acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it should usually be greater than one: this should avoid confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

2. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which concentrations of test material were properly measured at appropriate times during testing.

3. Results of chronic tests in which survival, growth, or reproduction in controls was unacceptably low should not be used. Limits of acceptability will depend on the species.

4. Results of chronic tests conducted in unusual dilution water should not be used, unless a relationship is developed between toxicity and the unusual characteristic or unless data show the characteristic does not affect toxicity.

5. Chronic values should be based on endpoints and exposure durations appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:

   a. Life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Tests with fish should begin with embryos or newly hatched young < 48 hr old, continue through maturation and reproduction, and should end not < 24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young < 24 hr old and last for not < 21 days. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female.

   b. Partial life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with juveniles at least 2 months prior to active gonadal development, continue through maturation and reproduction, and should end not < 24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.

   c. Early life-stage toxicity tests consisting of 28- to 32-day (60 days posthatch for salmonids) exposures of early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained on growth and survival.

      NOTE: Results of an early life-stage test are used as predictors of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used, because results of such tests may be poor estimates of results of a comparable life-cycle or partial life-cycle test.

6. A chronic value may be obtained by calculating the geometric mean of lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of an adverse effect on any specified biological measurements, and (c) below which no tested concentration caused such an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of an adverse effect on one or more of specified biological measurements, and (c) above which all tested concentrations caused such an effect.

7. If chronic toxicity of the material to aquatic animals appears to be related to a water quality characteristic, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section 8.

8. If chronic values are available for species in eight families as described in Section 4 (No. 1), a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values for the species, and appropriate Genus Mean Chronic Values should be calculated. The FCV should then be obtained using procedures described in Section 5 (Nos. 10-14). Then go to Section 7 (No. 13).

9. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. Acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water may be used, or acute tests conducted in the same dilution water but a different laboratory may be used. If such acute tests are not available, an acute-chronic ratio should not be calculated.

10. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios for that species.

11. For some materials the acute-chronic ratio is about the same for all species, but for other materials the ratio increases or decreases as the SMAV increases. Thus, the Final Acute-Chronic Ratio can be obtained in three ways, depending on the data.

    a. If the species mean acute-chronic ratio increases or decreases as the SMAV increases, the final Acute-Chronic Ratio should be

calculated as the geometric mean of all species whose SMAVs are close to the FAV.

b. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute--Chronic Ratio should be calculated as the geometric mean of all species mean acute-chronic ratios for both freshwater and saltwater species.

c. If the most appropriate species mean acute-chronic ratios are <2.0, and especially if they are < 1.0, acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be set at 2.0 so that the FCV is equal to the Criterion Maximum Concentration.

If the acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and an FCV probably cannot be calculated.

12. Calculate the FCV by dividing the FAV by the Final Acute-Chronic Ratio.

13. If the SMAV of an important species is lower than the calculated FCV, then that SMCV should be used as the FCV.

14. Go to Section 9.

8. FINAL CHRONIC EQUATION

1. A Final Chronic Equation can be derived in two ways. The procedure described in this section will result in the chronic slope being the same as the acute slope.

   a. If acute-chronic ratios for enough species at enough values of the water quality characteristics indicate that the acute-chronic ratio is probably the same for all species and independent of the water quality characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the species mean acute-chronic ratios.

   b. Calculate the FCV at the selected value Z of the water quality characteristic by dividing the FAV at Z (see Section 6, No. 13) by the Final Acute-Chronic Ratio.

   c. Use V = pooled acute slope (see Section 6, No. 13) as L = pooled chronic slope.

   d. Go to Section 8, No. 2, item m.

2. The procedure described in this section will usually result in the chronic slope being different from the acute slope.

   a. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

   b. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of chronic toxicity values on values of the water quality characteristic.

   c. Decide whether data for each species are useful, taking into account range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used. If a useful chronic slope is not available for at least one species or if the slopes are too dissimilar or if data are inadequate to define the relationship between chronic toxicity and water quality characteristic, return to Section 7 (No. 8), using results of tests conducted under conditions and in water similar to those commonly used for toxicity tests with the species.

   d. For each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species. This normalizes the chronic values so that the geometric mean of the normalized values for each species and for any combination of species is 1.0.

   e. Similarly normalize the values of the water quality characteristic for each species individually.

   f. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 1. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

   g. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope (L) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

h. For each species calculate the geometric mean (M) of toxicity values and the geometric mean (P) of related values of the water quality characteristic.

i. For each species calculate the logarithm (Q) of the SMCVs at a selected value (Z) of the water quality characteristic using the equation: $Q = \ln M - L(\ln P - \ln Z)$.

j. For each species calculate a SMCV at Z as the antilog of Q (SMCV $= e^{Q}$).

k. Obtain the FCV at Z by using the procedure described in Section 5 (Nos. 10-14).

l. If the SMCV at Z of an important species is lower than the calculated FCV at Z, then that SMCV should be used as the FCV at Z.

m. The Final Chronic Equation is written as:

$$FCV = e^{(L[\ln(\text{water quality characteristic})] + \ln S - L[\ln Z])},$$

where L = mean chronic slope and S = FCV at Z.

9. FINAL PLANT VALUE

1. Appropriate measures of toxicity of the material to aquatic plants are used to compare relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting results of toxicity tests with plants are not well developed, results of such tests usually indicate that criteria which adequately protect aquatic animals and their uses also protect aquatic plants and their uses.

2. A plant value is the result of any test conducted with an alga or an aquatic vascular plant.

3. Obtain the Final Plant Value by selecting the lowest result obtained in a test on an important aquatic plant species in which concentrations of test material were measured and the endpoint is biologically important.

10. FINAL RESIDUE VALUE

1. The Final Residue Value (FRV) is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from exceeding applicable FDA action levels and (b) protect wildlife, including fish and birds, that consume aquatic organisms from demonstrated unacceptable effects. The FRV is the lowest of residue values that are obtained by dividing maximum permissible tissue

concentrations by appropriate bioconcentration or bioaccumulation factors. A maximum permissible tissue concentration is either (a) an FDA action level (FDA administrative guidelines) for fish oil or for the edible portion of fish or shellfish or (b) a maximum acceptable dietary intake (ADI) based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to Section 11., because a Final Residue Value cannot be derived.

2. Bioconcentration Factors (BCFs) and Bioaccumulation Factors (BAFs) are the quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution to which the organism has been exposed. A BCF is intended to account only for net uptake directly from water, and thus almost has to be measured in a laboratory test. A BAF is intended to account for net uptake from both food and water in a real-world situation, and almost has to be measured in a field situation in which predators accumulate the material directly from water and by consuming prey. Because so few acceptable BAFs are available, only BCFs will be discussed further, but an acceptable BAF can be used in place of a BCF.

3. If a maximum permissible tissue concentration is available for a substance (e.g, parent material or parent material plus metabolite), the tissue concentration used in BCF calculations should be for the same substance. Otherwise the tissue concentration used in the BCF calculation should be that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.

   a. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of test material in tissue and in the test solution, and exposure continued at least until either apparent steady-state (BCF does not change significantly over a period of time, such as two days or 16 percent of exposure duration, whichever is longer) or 28 days was reached. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached; (b) highest BCF obtained, if apparent steady-state was not reached; and (c) projected steady-state BCF, if calculated.

   b. Whenever a BCF is determined for a lipophilic material, percentage of lipids should also be determined in the tissue(s) for which the BCF is calculated.

   c. A BCF obtained from an exposure that adversely affected the test organisms may be used only if it is similar to that obtained with unaffected individuals at lower concentrations that did cause effects.

d. Because maximum permissible tissue concentrations are rarely based on dry weights, a BCF calculated using dry tissue weights must be converted to a wet tissue weight basis. If no conversion factor is reported with the BCF, multiply the dry weight by 0.1 for plankton and by 0.2 for species of fishes and invertebrates.

e. If more than one acceptable BCF is available for a species, the geometric mean of values should be used, unless the BCFs are from different exposure durations, in which case the BCF for the longest exposure should be used.

4. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:

   a. For each available maximum ADI derived from a feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of tested wildlife species.

   b. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.

5. For lipophilic materials, it may be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percentage of lipids from one tissue to another and from one species to another (Hamelink et al. 1971, Lundsford and Blem 1982, Schnoor 1982), extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percentage of lipids.

   a. For each BCF for which percentage of lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by percentage of lipids. This adjustment makes all the measured BCFs comparable regardless of species or tissue.

   b. Calculate the geometric mean normalized BCF.

   c. Calculate all possible residue values by dividing available maximum permissible tissue concentrations by the mean normalized BCF and by the percentage of lipids values appropriate to the maximum permissible tissue concentration.

      • For an FDA action level for fish oil, the appropriate percentage of lipids value is 100.

- For an FDA action level for fish, the appropriate percentage of lipids value is 11 for freshwater criteria, based on the highest levels for important consumed species (Sidwell 1981).

- For a maximum ADI derived from a chronic feeding study or long-term field study with wildlife, the appropriate percentage of lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.

6. The FRV is obtained by selecting the lowest of available residue values.

11. OTHER DATA

Pertinent information that could not be used in earlier sections may be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other biologically important adverse effect. Especially important are data for species for which no other data are available.

12. CRITERION

1. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.

2. The Criterion Maximum Concentration (CMC) is equal to one-half of the FAV.

3. The Criterion Continuous Concentration (CCC) is equal to the lowest of the FCV, the Final Plant Value, and the FRV unless other data show a lower value should be used. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final Chronic Equation, the Final Plant Value, and the FRV by selecting the value or concentration that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section 11) show that a lower value should be used.

4. Round both the CCC and CMC to two significant figures.

5. The criterion is stated as: The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that (except possibly where a locally important species is very sensitive) (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) $\mu g/L$ more than once every three years on the average and if the one-hour average concentration does not exceed (4) $\mu g/L$ more than once every three years on the average.

Where,

(1) = insert freshwater or saltwater,
(2) = insert name of material,
(3) = insert the Criterion Continuous Concentration, and
(4) = insert the Criterion Maximum Concentration.

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APPENDIX B

SUMMARY OF USEPA METHODOLOGY FOR DETERMINING WATER QUALITY CRITERIA FOR THE PROTECTION OF HUMAN HEALTH

The following summary is a condensed version of the 1980 final U.S. Environmental Protection Agency (USEPA) guidelines for calculating water quality criteria to protect human health and is slanted towards the specific regulatory needs of the U.S. Army. The guidelines are the most recent document outlining the required procedures and were published in the Federal Register (USEPA 1980). For greater detail on individual points consult that reference.

1. INTRODUCTION

The EPA's water quality criteria for the protection of human health are based on one or more of the following properties of a chemical pollutant:

(a) Carcinogenicity, (b) Toxicity, and (c) Organoleptic (taste and odor) effects.

The meanings and practical uses of the criteria values are distinctly different depending on the properties on which they are based. Criteria based solely on organoleptic effects do not necessarily represent approximations of acceptable risk levels for human health. In all other cases the values represent estimates that would prevent adverse health effects or, for suspect and proven carcinogens, estimations of the increased cancer risk associated with incremental changes in the ambient water concentration of the substance. Social and economic costs and benefits are not considered in determining water quality criteria. In establishing water quality standards, the choice of the criterion to be used depends on the designated water use. In the case of a multiple-use water body, the criterion protecting the most sensitive use is applied.

2. DATA NEEDED FOR HUMAN HEALTH CRITERIA

Criteria documentation requires information on: (1) exposure levels, (2) pharmacokinetics, and (3) range of toxic effects of a given water pollutant.

2.1 EXPOSURE DATA

For an accurate assessment of total exposure to a chemical, consideration must be given to all possible exposure routes including ingestion of contaminated water and edible aquatic and nonaquatic organisms, as well as exposure through inhalation and dermal contact. For water quality criteria the most important exposure routes to be considered are ingestion of water and consumption of fish and shellfish. Generally,

exposure through inhalation, dermal contact, and non-aquatic diet is either unknown or so low as to be insignificant: however, when such data are available, they must be included in the criteria evaluation.

The EPA guidelines for developing water quality criteria are based on the following assumptions, which are designed to be protective of a healthy adult male who is subject to average exposure conditions:

1. The exposed individual is a 70-kg male person (International Commission on Radiological Protection 1977).

2. The average daily consumption of freshwater and estuarine fish and shellfish products is equal to 6.5 grams.

3. The average daily ingestion of water is equal to 2 liters (Drinking Water and Health, National Research Council 1977).

Because fish and shellfish consumption is an important exposure factor, information on bioconcentration of the pollutant in edible portions of ingested species is necessary to calculate the overall exposure level. The bioconcentration factor (BCF) is equal to the quotient of the concentration of a [illegible]tance in all or part of an organism divided by the concentration in a[illegible] water to which the organism has been exposed. The BCF is a func[illegible]f lipid solubility of the substance and relative amount of lipids [illegible]edible portions of fish or shellfish. To determine the weighted average BCF, three different procedures can be used depending upon lipid solubility and availability of bioconcentration data:

1. For lipid soluble compounds, the average BCF is calculated from the weighted average percent lipids in ingested fish and shellfish in the average American diet. The latter factor has been estimated to be 3 percent (Stephan 1980, as cited in USEPA 1980). Because steady-state BCFs for lipid soluble compounds are proportional to percent lipids, the BCF for the average American diet can be calculated as follows:

$$BCF_{avg} = BCF_{sp} \times \frac{3.0\%}{PL_{sp}},$$

where BCF_{sp} is the bioconcentration factor for an aquatic species and PL_{sp} is the percent lipids in the edible portions of that species.

2. Where an appropriate bioconcentration factor is not available, the BCF can be estimated from the octanol/water partition coefficient (P) of a substance as follows:

$$\log BCF = (0.85 \log P) - 0.70$$

for aquatic organisms containing about 7.6 percent lipids (Veith et al. 1980, as cited in USEPA 1980). An adjustment for percent lipids in the average diet (3 percent versus 7.6 percent) is made to derive the weighted average bioconcentration factor.

3. For nonlipid-soluble compounds, the available BCFs for edible portions of consumed freshwater and estuarine fish and shellfish are weighted according to consumption factors to determine the weighted BCF representative of the average diet.

2.2 PHARMACOKINETIC DATA

Pharmacokinetic data, encompassing information on absorption, distribution, metabolism, and excretion, are needed for determining the biochemical fate of a substance in human and animal systems. Information on absorption and excretion in animals, together with a knowledge of ambient concentrations in water, food, and air, are useful in estimating body burdens in humans. Pharmacokinetic data are also essential for estimating equivalent oral doses based on data from inhalation or other routes of exposure.

2.3 BIOLOGICAL EFFECTS DATA

Effects data which are evaluated for water quality criteria include acute, subchronic, and chronic toxicity: synergistic and antagonistic effects; and genotoxicity, teratogenicity, and carcinogenicity. The data are derived primarily from animal studies, but clinical case histories and epidemiological studies may also provide useful information. According to the EPA (USEPA 1980), several factors inherent in human epidemiological studies often preclude their use in generating water quality criteria (see NAS 1977). However, epidemiological data can be useful in testing the validity of animal-to-man extrapolations

From an assessment of all the available data, a biological endpoint, i.e., carcinogenicity, toxicity, or organoleptic effects, is selected for criteria formulation.

3. HUMAN HEALTH CRITERIA FOR CARCINOGENIC SUBSTANCES

If sufficient data exist to conclude that a specific substance is a potential human carcinogen (carcinogenic in animal studies, with supportive genotoxicity data, and possibly also supportive epidemiological data) then the position of the EPA is that the water quality criterion for that substance (recommended ambient water concentration for maximum protection of human health) is zero. This is because the EPA believes that no method exists for establishing a threshold level for carcinogenic effects, and, consequently, there is no scientific basis for establishing a "safe" level. To better define the carcinogenic risk associated with a

particular water pollutant, the EPA has developed a methodology for determining ambient water concentrations of the substance which would correspond to incremental lifetime cancer risks of 10^{-7} to 10^{-5} (one additional case of cancer in populations ranging from ten million to 100,000, respectively). These risk estimates, however, do not represent an EPA judgment as to an "acceptable" risk level.

3.1. METHODOLOGY FOR DETERMINING CARCINOGENICITY (NONTHRESHOLD) CRITERIA

The ambient water concentration of a substance corresponding to a specific lifetime carcinogenic risk can be calculated as follows:

$$C = \frac{70 \times PR}{q_1^* (2 + 0.0065\ BCF)}$$

where

C = ambient water concentration;
PR = the probable risk (e.g., 10^{-5}; equivalent to one case in 100,000);
BCF = the bioconcentration factor; and
q_1^* = a coefficient, the cancer potency index (defined below) (USEPA 1980).

By rearranging the terms in this equation, it can be seen that the ambient water concentration is one of several factors which define the overall exposure level:

$$PR = \frac{q_1^* \times C \ (2 + 0.0065\ BCF)}{70}$$

or

$$PR = \frac{q_1^* \times 2C + (0.0065\ BCF \times C)}{70}$$

where

2C is the daily exposure resulting from drinking 2 liters of water per day and (0.0065 BCF x C) is the average daily exposure resulting from the consumption of 6.5 mg of fish and shellfish per day. Because the exposure is calculated for a 70-kg man, it is normalized to a per

kilogram basis by the factor of 1/70. In this particular case, exposure resulting from inhalation, dermal contact, and nonaquatic diet is considered to be negligible.

In simplified terms the equation can be rewritten

$$PR = q_1^* \ X \ ,$$

where X is the total average daily exposure in mg/kg/day or

$$q_1^* = \frac{PR}{X} \ ,$$

showing that the coefficient q_1^* is the ratio of risk to dose, an indication of the carcinogenic potency of the compound.

The USEPA guidelines state that for the purpose of developing water quality criteria, the assumption is made that at low dose levels there is a linear relationship between dose and risk (at high doses, however, there may be a rapid increase in risk with dose resulting in a sharply curved dose/response curve). At low doses then, the ratio of risk to dose does not change appreciably and q_1^* is a constant. At high doses the carcinogenic potency can be derived directly from experimental data, but for risk levels of 10^{-7} to 10^{-5}, which correspond to very low doses, the q_1^* value must be derived by extrapolation from epidemiological data or from high dose, short-term animal bioassays.

3.2 CARCINOGENIC POTENCY CALCULATED FROM HUMAN DATA

In human epidemiological studies, carcinogenic effect is expressed in terms of the relative risk [RR(X)] of a cohort of individuals at exposure X compared with the risk in the control group [PR(control)] (e.g., if the cancer risk in group A is five times greater than that of the control group, then RR(X) = 5). In such cases the "excess" relative cancer risk is expressed as RR(X) - 1, and the actual numeric, or proportional, excess risk level [PR(X)] can be calculated:

$$PR(X) = [RR(X) - 1] \times PR(control).$$

Using the standard risk/dose equation

$$PR(X) = b \times X$$

and substituting for PR(X):

$$[RR(X) - 1] \times PR(control) = b \times X$$

or

$$b = \frac{[RR(X) - 1] \times PR(control)}{X}$$

where b is equal to the carcinogenic potency or q_1^*.

3.3 CARCINOGENIC POTENCY CALCULATED FROM ANIMAL DATA

In the case of animal studies where different species, strains, and sexes may have been tested at different doses, routes of exposure, and exposure durations, any data sets used in calculating the health criteria must conform to certain standards:

1. The tumor incidence must be statistically significantly higher than the control for at least one test dose level and/or the tumor incidence rate must show a statistically significant trend with respect to dose level.

2. The data set giving the highest index of cancer potency (q_1^*) should be selected unless the sample size is quite small and another data set with a similar dose-response relationship and larger sample size is available.

3. If two or more data sets are comparable in size and identical with respect to species, strain, sex, and tumor site, then the geometric mean of q_1^* from all data sets is used in the risk assessment.

4. If in the same study tumors occur at a significant frequency at more than one site, the cancer incidence is based on the number of animals having tumors at any one of those sites.

In order to make different data sets comparable, the EPA guidelines call for the following standardized procedures:

1. To establish equivalent doses between species, the exposures are normalized in terms of dose per day (m) per unit of body surface area. Because the surface area is proportional to the 2/3 power of the body weight (W), the daily exposure (X) can be expressed as:

$$X = \frac{m}{W^{2/3}}$$

2. If the dose (s) is given as mg per kg of body weight:

$$s = \frac{m}{W}$$

then

$$m = s \times W$$

and the equivalent daily exposure (X) would be

$$X = \frac{(s \times w)}{W^{2/3}}$$

or

$$X = s \times W^{1/3} .$$

3. The dose must also be normalized to a lifetime average exposure. For a carcinogenic assay in which the average dose per day (in mg) is m, and the length of exposure is l_e, and the total length of the experiment is Le, then the lifetime average exposure (X_m) is

$$X_m = \frac{l_e \times m}{L_e \times W^{2/3}}$$

4. If the duration of the experiment (L_e) is less than the natural life span (L) of the test animal, the value of q_1^* is increased by a factor of $(L/L_e)3$ to adjust for an age-specific increase in the cancer rate.

5. If the exposure is expressed as the dietary concentration of a substance (in ppm), then the dose per day (m) is

$$m = ppm \times F \times r ,$$

where F is the weight of the food eaten per day in kg, and r is the absorption fraction (which is generally assumed to be equal to 1). The weight of the food eaten per day can be expressed as a function of body weight

$$F = fW ,$$

where f is a species-specific, empirically derived coefficient which adjusts for differences in F due to differences in the caloric content of each species diet (f is equal to 0.028 for a 70-kg man: 0.05 for a 0.35-kg rat: and 0.13 for a 0.03-kg mouse).

Substituting (ppm x F) for m and fW for F, the daily exposure (dose/surface area/day or $m/W^{2/3}$) can be expressed as

$$X = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times f \times W}{W^{2/3}} = ppm \times f \times W^{1/3}$$

6. When exposure is via inhalation, calculation can be considered for two cases: (1) the substance is a water soluble gas or aerosol and is absorbed proportionally to to the amount of air breathed in and (2) the substance is not very water soluble and absorption, after equilibrium is reached between the air and the body compartments, will be proportional to the metabolic rate which is proportional to rate of oxygen consumption, which, in turn, is a function of total body surface area.

3.4 EXTRAPOLATION FROM HIGH TO LOW DOSES

Once experimental data have been standardized in terms of exposure levels, they are incorporated into a mathematical model which allows for calculation of excess risk levels and carcinogenic potency at low doses by extrapolation from high dose situations. There are a number of mathematical models which can be used for this procedure (see Krewski et al. 1983 for review). The EPA has selected a "linearized multi-stage" extrapolation model for use in deriving water quality criteria (USEPA 1980). This model is derived from a standard "general product" time-to-response (tumor) model (Krewski et al. 1983):

$$P(t;d) = 1 - \exp(-g(d)H(t)) ,$$

where P(t;d) is the probable response for dose d and time t, g(d) is the polynomial function defining the effect of dose level, and H(t) is the effect of time:

$$g(d) = \sum_{i=0}^{a} \alpha_i d^i$$

$$H(t) = \sum_{i=0}^{b} \beta_i t^i$$

(with α and $\beta \geq 0$, and $\Sigma \beta_i = 1$).

This time-to-response model can be converted to a quantal response model by incorporation of the time factor into each α as a multiplicative constant (Crump 1980):

$$p(d/t) = 1 - \exp\left(- \sum_{i=0}^{a} \alpha d^i \right),$$

or as given in the EPA guidelines (USEPA 1980):

$$p(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

where P(d) is the lifetime risk (probability) of cancer at dose d.

For a given dose the excess cancer risk A(d) above the background rate P(o) is given by the equation:

$$A(d) = \frac{P(d) - P(o)}{1 - P(o)}$$

where

$$A(d) = 1 - \exp[-q_1 d + q_2 d^2 + \ldots + q_k d^k)].$$

Point estimates of the coefficients $q_1 \ldots q_k$ and consequently the extra risk function A(d) at any given dose are calculated by using the statistical method of maximum likelihood. Whenever q_1 is not equal to 0, at low doses the extra risk function A(d) has approximately the form:

$$A(d) = q_1 \times d.$$

Consequently, $q_1 \times d$ represents a 95 percent upper confidence limit on the excess risk, and R/q_1 represents a 95 percent lower confidence limit on the dose producing an excess risk of R. Thus A(d) and R will be a function of the maximum possible value of q_1 which can be determined from the 95 percent upper confidence limits on q_1. This is accomplished by

using the computer program GLOBAL 79 developed by Crump and Watson (1979). In this procedure q_1^*, the 95 percent upper confidence limit, is calculated by increasing q_1 to a value which, when incorporated into the log-likelihood function, results in a maximum value satisfying the equation:

$$2(L_0 - L_1) = 2.70554,$$

where L_0 is the maximum value of the log-likelihood function.

Whenever the multistage model does not fit the data sufficiently, data at the highest dose are deleted and the model is refitted to the data. To determine whether the fit is acceptable, the chi-square statistic is used:

$$x^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i (1 - P_i)}$$

where N_i is the number of animals in the ith dose group, X_i is the number of animals in the ith dose group with a tumor response, P_i is the probability of a response in the ith dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups.

The fit is determined to be unacceptable whenever chi-square (x^2) is larger than the cumulative 99 percent point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of nonzero multistage coefficients.

4. HEALTH CRITERIA FOR NONCARCINOGENIC TOXIC SUBSTANCES

Water quality criteria that are based on noncarcinogenic human health effects can be derived from several sources of data. In all cases it is assumed that the magnitude of a toxic effect decreases as the exposure level decreases until a threshold point is reached at and below which the toxic effect will not occur regardless of the length of the exposure period. Water quality criteria (C) establish the concentration of a substance in ambient water which, when considered in relation to other sources of exposure [i.e., average daily consumption of nonaquatic organisms (DT) and daily inhalation (IN)], place the Acceptable Daily Intake (ADI) of the substance at a level below the toxicity threshold, thereby preventing adverse health effects:

$$C = \frac{ADI \cdot (DT + IN)}{[2L + (0.0065\ kg \times BCF)]}$$

where 2L is the amount of water ingested per day, 0.0065 kg is the amount of fish and shellfish consumed per day, and BCF is the weighted average bioconcentration factor.

In terms of scientific validity, an accurate estimate of the ADI is the major factor in deriving a satisfactory water quality criterion.

The threshold exposure level, and thus the ADI, can be derived from either or both animal and human toxicity data.

4.1 NONCARCINOGENIC HEALTH CRITERIA BASED ON ANIMAL TOXICITY DATA (ORAL)

For criteria derivation, toxicity is defined as any adverse effects which result in functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (USEPA 1980).

A bioassay yielding information as to the highest chronic (90 days or more) exposure tolerated by the test animal without adverse effects (No-Observed-Adverse-Effect-Level or NOAEL) is equivalent to the toxicity threshold and can be used directly for criteria derivation. In addition to the NOAEL, other data points which can be obtained from toxicity testing are

(1) NOEL = No-Observed-Effect-Level,
(2) LOEL = Lowest-Observed-Effect-Level,
(3) LOAEL = Lowest-Observed-Adverse-Effect-Level,
(4) FEL = Frank-Effect-Level.

According to the EPA guidelines, only certain of these data points can be used for criteria derivation:

1. A single FEL value, without information on the other response levels, should not be used for criteria derivation because there is no way of knowing how far above the threshold it occurs.

2. A single NOEL value is also unsuitable because there is no way of determining how far below the threshold it occurs. If only multiple NOELs are available, the highest value should be used.

3. If an LOEL value alone is available, a judgement must be made as to whether the value actually corresponds to an NOAEL or an LOAEL.

4. If an LOAEL value is used for criteria derivation, it must be adjusted by a factor of 1 to 10 to make it approximately equivalent to the NOAEL and thus the toxicity threshold.

5. If for reasonably closely spaced doses only an NOEL and an LOAEL value of equal quality are available, the NOEL is used for criteria derivation.

The most reliable estimate of the toxicity threshold would be one obtained from a bioassay in which an NOEL, an NOAEL, an LOAEL, and a clearly defined FEL were observed in relatively closely spaced doses.

Regardless of which of the above data points is used to estimate the toxicity threshold, a judgement must be made as to whether the experimental data are of satisfactory quality and quantity to allow for a valid extrapolation for human exposure situations. Depending on whether the data are considered to be adequate or inadequate, the toxicity threshold is adjusted by a "safety factor" or "uncertainty factor" (NAS 1977). The "uncertainty factor" may range from 10 to 1000 according to the following general guidelines:

1. Uncertainty factor 10. Valid experimental results from studies on prolonged ingestion by man, with no indication of carcinogenicity.

2. Uncertainty factor 100. Data on chronic exposures in humans not available. Valid results of long-term feeding studies on experimental animals, or in the absence of human studies, valid animal studies on one or more species. No indication of carcinogenicity.

3. Uncertainty factor 1000. No long-term or acute exposure data for humans. Scanty results on experimental animals, with no indication of carcinogenicity.

Uncertainty factors which fall between the categories described above should be selected on the basis of a logarithmic scale (e.g., 33 being halfway between 10 and 100).

The phrase "no indication of carcinogenicity" means that carcinogenicity data from animal experimental studies or human epidemiology are not available. Data from short-term carcinogenicity screening tests may be reported, but they are not used in criteria derivation or for ruling out the uncertainty factor approach.

4.2 CRITERIA BASED ON INHALATION EXPOSURES

In the absence of oral toxicity data, water quality criteria for a substance can be derived from threshold limit values (TLVs) established by the American Conference of Governmental and Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA), or the National Institute for Occupational Safety and Health (NIOSH), or from laboratory studies evaluating the inhalation toxicity of the substance in experimental animals. TLVs represent 8-hr time-weighted averages of concentrations in air designed to protect workers from various adverse

health effects during a normal working career. To the extent that TLVs are based on sound toxicological evaluations and have been protective in the work situation, they provide helpful information for deriving water quality criteria. However, each TLV must be examined to decide if the data it is based on can be used for calculating a water quality criterion (using the uncertainty factor approach). Also the history of each TLV should be examined to assess the extent to which it has resulted in worker safety. With each TLV, the types of effects against which it is designed to protect are examined in terms of its relevance to exposure from water. It must be shown that the chemical is not a localized irritant and there is no significant effect at the portal of entry, regardless of the exposure route.

The most important factor in using inhalation data is in determining equivalent dose/response relationships for oral exposures. Estimates of equivalent doses can be based upon (1) available pharmacokinetic data for oral and inhalation routes, (2) measurements of absorption efficiency from ingested or inhaled chemicals, or (3) comparative excretion data when associated metabolic pathways are equivalent to those following oral ingestion or inhalation. The use of pharmacokinetic models is the preferred method for converting from inhalation to equivalent oral doses.

In the absence of pharmacokinetic data, TLVs and absorption efficiency measurements can be used to calculate an ADI value by means of the Stokinger and Woodward (1958) model:

$$ADI = \frac{TLV \times BR \times DE \times d \times A_A}{(A \times SF)_O}$$

where

BR = daily air intake (assume 10 m^3),
DE = duration of exposure in hours per day,
d = 5 days/7 days,
A_A = efficiency of absorption from air,
A_O = efficiency of absorption from oral exposure, and
SF = safety factor.

For deriving an ADI from animal inhalation toxicity data, the equation is:

$$ADI = \frac{C_A \times D_E \times d \times A_A \times BR \times 70\ kg}{(BW_A \times A_O \times SF)}$$

where

C_A = concentration in air (mg/m3),
D_E = duration of exposure (hr/day),
d = number of days exposed/number of days observed,
A_A = efficiency of absorption from air,

BR - volume of air breathed (m3/day),
70 kg - standard human body weight,
BW_A - body weight of experimental animals (kg),
A_O - efficiency of absorption from oral exposure, and
SF - safety factor.

The safety factors used in the above equations are intended to account for species variability. Consequently, the mg/surface area/day conversion factor is not used in this methodology.

5. ORGANOLEPTIC CRITERIA

Organoleptic criteria define concentrations of substances which impart undesirable taste and/or odor to water. Organoleptic criteria are based on aesthetic qualities alone and not on toxicological data, and therefore have no direct relationship to potential adverse human health effects. However, sufficiently intense organoleptic effects may, under some circumstances, result in depressed fluid intake which, in turn, might aggravate a variety of functional diseases (i.e., kidney and circulatory diseases).

For comparison purposes, both organoleptic criteria and human health effects criteria can be derived for a given water pollutant; however, it should be explicitly stated in the criteria document that the organoleptic criteria have no demonstrated relationship to potential adverse human health effects.

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1	CHIEF USAEHA REGIONAL DIVISION, NORTH FORT GEORGE G. MEADE, MD 20755
1	CHIEF USAEHA REGIONAL DIVISION, SOUTH BLDG. 180 FORT MCPHERSON, GA 30330
1	COMMANDER USA HEALTH SERVICES COMMAND ATTN: HSPA-P FORT SAM HOUSTON, TX 78234

1 COMMANDANT
ACADEMY OF HEALTH SCIENCES
UNITED STATES ARMY
ATTN: CHIEF, ENVIRONMENTAL QUALITY BRANCH
PREVENTIVE MEDICINE DIVISION (HSHA-IPM)
FORT SAM HOUSTON, TX 78234

1 COMMANDER US ARMY MATERIEL COMMAND
ATTN: AMSCG
5001 EISENHOWER AVENUE
ALEXANDER, VA 22333

1 COMMANDER
US ARMY ENVIRONMENTAL HYGIENE AGENCY
ATTN: LIBRARIAN, HSDH-AD-L
ABERDEEN PROVING GROUND, MD 21010

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1 HQDA
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FALLS CHURCH, VA 22041-3258

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ATTN: DAEN-RDM
20 MASSACHUSETTS, NW
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